INDEX NUMBER

Journal of
DENTAL
RESEARCH

Founded by William J. Gies in 1919

OFFICIAL PUBLICATION OF
THE INTERNATIONAL ASSOCIATION
FOR DENTAL RESEARCH

UNIVERSITY OF MICHIGAN
DENTISTRY LIBRARY

NOVEMBER—DECEMBER, 1958

Editorial Views and News ---------- 989

International Association for Dental Research Announcement of 1959 General Meeting ---------- 990

A Study of the Inorganic Carbon Dioxide in Intact Human Enamel. M. F. Little and Finn Brudevold, Rochester, N. Y. ---------- 991

Further Studies on the Action of Collagenase on Sound and Carious Human Dentin. W. G. Armstrong, London, W.C. 1, England ---------- 1001

Modification of the Organic Matrix of Sound Dentin to Collagenase-Resistant Forms. W. G. Armstrong, London, W.C. 1, England ---------- 1016

Continuous Cementum Apposition. H. A. Zander and Beat Hürzeler, Rochester, N. Y. ---------- 1035

Chemical and Physical Studies of Enamel From Human Teeth. II. Specific Gravity, Nitrogen Content, and Hardness Rating of Discolored Enamel. B. R. Bhussry, Rochester, N. Y. ---------- 1045

(Contents continued on inside back cover)

. 37 No. 6

Editor
FRANK J. ORLAND

Published for
THE INTERNATIONAL
ASSOCIATION FOR
DENTAL RESEARCH

By
THE C. V. MOSBY COMPANY
ST. LOUIS 3, MO.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

THE JOURNAL OF DENTAL RESEARCH

Editor—Frank J. Orland.

Assistant to the Editor—Vernon J. Brightman.

Manuscript Secretary—Dorothy R. Herbison.

Associate Editors—David F. Mitchell and John B. Macdonald.

Publication Committee—N. B. Williams, H. B. G. Robinson, D. F. Mitchell, J. B. Macdonald, D. Y. Burrill, *ex officio*, F. J. Orland, *chairman*.

ADVISORY EDITORIAL BOARD

J. K. Avery	S. J. Kreshover	S. Pearlman	D. B. Scott
F. Brudevold	W. Lefkowitz	R. W. Phillips	W. G. Shafer
G. W. Burnett	S. W. Leung	J. Pindborg	M. V. Stack
H. C. Hodge	B. M. Levy	A. L. Russell	W. W. Wainwright
H. A. Hunter	R. S. Manly	L. H. Schneyer	

DIVISIONS AND SECTIONS

Division	*Section*	*President or Chairman*	*Secretary*	*Councillor*
North American	Ann Arbor	J. K. Avery	K. Asgar	M. C. Crowley
	Birmingham	R. C. Caldwell	W. W. Pigman	G. Parfitt
	Boston	C. F. A. Moorrees	P. Goldhaber	P. L. Munson
	Chicago	S. Pearlman	V. E. James	L. Fosdick
	Cleveland		S. W. Chase	T. J. Hill
	Columbus		S. Kolas	W. Lefkowitz
	Houston	B. Levy	D. A. Hagerman	S. S. Arnim
	Indianapolis	R. W. Phillips	M. L. Swartz	J. C. Muhler
	Iowa City	E. Hixon	D. E. Waite	A. K. Fisher
	Minnesota	L. Singer	R. Gorlin	J. Gibilisco
	New York	H. J. Bartelstone	E. D. Goldsmith	G. Bevelander
	Pacific Northwest	B. S. Savara	D. M. Hadjimarkos	L. Sreebny
	Philadelphia	I. B. Bender	L. Baumann	L. I. Grossman
	Rochester, N. Y.	R. Lobene	E. Johansen	B. G. Bibby
	St. Louis		J. R. Ring	T. Rosebury
	San Francisco		S. Epstein	W. C. Fleming
	Southern California			B. Z. Rabinowitch
	Toronto	R. C. Burgess	D. L. Anderson	G. Nikiforuk
	Washington, D. C.	G. Brauer	J. W. Stanford	J. A. English
British			C. H. Tonge	M. A. Rushton
Scandinavia	Denmark	P. O. Pedersen	J. J. Pindborg	J. J. Holst
	Norway	G. Toverud	I. Reichborn-Kjennerud	
Not belonging to a Division	Helsinki	E. Tammisalo	K. Koski	
	Japan	S. Takahashi	K. Enoki	G. Minoguchi
	Johannesburg	J. T. Irving	B. Cohen	A. J. Clement
	Mexico		F. R. Leycegui	
	Palestine		S. Lewin-Epstein	
	Prague		F. Neumann	
	Austria, Vienna Center		E. Zitka	F. Driak
Dental Materials Group		G. Ryge	D. B. Mahler	H. D. Coy

COUNCILLORS-AT-LARGE

R. L. Ireland H. J. Merkeley

OFFICERS OF THE ASSOCIATION

President—Ned B. Williams (University of Pennsylvania), *President-Elect*—Hamilton B. G. Robinson (Ohio State University), *Vice-President*—Holmes T. Knighton (Medical College of Virginia), *Honorary Vice-President*—Edgar D. Coolidge, *Secretary-Treasurer*—Dan Y. Burrill (Northwestern University), *Assistant Secretary-Treasurer*—Edward H. Hatton (Northwestern University), *Editor*—Frank J. Orland (University of Chicago).

Vol. 37, No. 6, November-December, 1958. Journal of Dental Research is published bi-monthly by The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, Mo. Subscription rates: United States and its Possessions $10.00, Students $5.00; Canada, Latin-America, and Spain $10.50, Students $5.50; Other Countries $11.00, Students $6.00. Single copies $2.50 postpaid. Entered as second-class matter June 24, 1919, at the Post Office at St. Louis, Mo., under the Act of March 3, 1879. Printed in the U. S. A. Copyright © 1958 by The International Association for Dental Research.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Organized: New York City, Dec. 10, 1920. Of those at the meeting of organization, the following remain active members: Adolph Berger, Theodor Blum, G. W. Clapp, H. S. Dunning, A. H. Merritt, B. B. Palmer, L. M. Waugh.

Objects (Const., Art. II): The association has been established to promote broadly the advancement of research in all branches of dental science and in the related phases of the sciences that contribute directly to the development of oral health service, and as a whole; and further, to encourage and facilitate cooperative effort and achievement by, and mutual helpfulness among, investigators in all nations in every division of stomatology to the end that dentistry may render cumulatively more perfect service to humanity.

Membership: Total (October, 1958), 938. *Eligibility (Const., Art. III, Sec. 1).*—"Any person who has conducted, and published an account of, a meritorious original investigation in dental science, or in any of the sciences contributory to oral health, shall be eligible to membership in this Association, providing such person conforms to the recognized standards of professional ethics." *Election.*—New members may be elected *only at general meetings of the Association.* Membership in a section is secondary to membership in the Association as a whole. Removal of members from one geographic center to another does not affect their membership in the Association.

Nomination blanks for membership in this Association may be obtained on request from the Secretary-Treasurer.

Endowment fund of the International Association for Dental Research (as of October, 1958): $1,891.01. There have been no expenditures from this fund.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
37th General Meeting
Sheraton-Palace, San Francisco, California
March 19-22, 1959
For further instructions, contact Dr. Burrill, Sec.-Treas.

THE JOURNAL OF DENTAL RESEARCH

This JOURNAL is an official publication of the International Association for Dental Research. It is dedicated to the dissemination of knowledge pertaining to the oral cavity, its contents and contiguous parts, and its relation to and effect on the total human organism. The JOURNAL publishes original investigations in these and related fields. Articles are accepted only if they are contributed solely to this JOURNAL.

The JOURNAL is published bimonthly. Subscription price for the volume (6 copies beginning with the January-February issue) is as follows: United States and its possessions, $10.00; (Students, $5.00). Canada, Latin America and Spain, $10.50; (Students, $5.50). Other countries $11.00; (Students, $6.00). Single copies $2.50, postpaid. The subscription fee for members of the IADR is included in the yearly dues paid to the Association. The price of volumes and single numbers of earlier years will be quoted upon request to Dr. David F. Mitchell, University of Indiana, 1121 W. Michigan St., Indianapolis, Indiana.

Matters pertaining to subscriptions, lost copies, change of address, reprints (up to 500), cost of colored reproductions, advertising space, and other business matters should be addressed to The C. V. Mosby Company, Publishers, 3207 Washington Blvd., St. Louis, Missouri.

EDITORIAL VIEWS AND NEWS

This November-December issue completes Volume 37 of the JOURNAL and the year 1958. It has been the consensus of both Publisher and Editor that each number should carry a dual month heading so that there is obviated the not infrequent question as to what happened to "that alternate month's issue of the JOURNAL." Several other bimonthly publications have been changing their journal calender headings in like manner.

The AAAS Meeting.—Ordinarily the JOURNAL does not publish announcements of scientific meetings since mention is made of such gatherings by other available publications, and because there are so many, as indicated by the vast number of bulletins received by the JOURNAL Office, for which the Editor thanks the writers. However, in the case of the meeting of the American Association for the Advancement of Science, Section Nd, which is co-sponsored by the International Association for Dental Research, North American Division, the program is as follows: There will be a Symposium on "Calcification in Biological Systems" in Washington, D. C. (Hotel Shoreham), December 29. This highly significant Symposium will be co-sponsored also by the American Dental Association, the American College of Dentists, as well as by the AAAS Sections on Medicine (N) and Zoology (F).

It is interesting to observe that three of the guiding figures in this program are former Presidents of the IADR. Russell W. Bunting is Secretary, while George C. Paffenbarger is Vice-President and Chairman of Section Nd, AAAS, and Reidar F. Sognnaes is Chairman of the Symposium per se.

The expository papers to be presented in three sessions and their authors are listed in this order on the program:

1. Histogenesis and Histochemistry of the Exoskeleton of Molluscs and Echinoderms—Gerrit Bevelander (NYU).
2. Shell Structure and Mineralization in the Oyster *(Crassostrea virginica)*—Karl M. Wilbur (Duke).
3. Matrix and Mineral Deposition in the Skeleton of the Spiny Lobster *(Panulirus argus Latreille)* and the Gastrolith of the Crayfish *(Orconectes vivilis Hagen)*—Dorothy F. Travis (Harvard).
4. Histochemical Matrix Reactions Accompanying the Calcium Carbonate Deposition in Rodent Otoliths—Leonard F. Belanger (Ottawa).
5. Calcification of Cartilage—Richard H. Follis, Jr. (AFIP).
6. Histological, Physical and Chemical Studies on the Mineralizing Turkey Leg-Tendon—Robert C. Likins, Marie U. Nylen, Karl A. Piez, David B. Scott (NIDR); Vernon M. Mosley and Lent C. Johnson (AFIP).
7. Enzymatic Reactions in Osteogenesis and Odontogenesis—Marvin S. Burstone (NIDR).
8. Dental Aspects of Mineralization With Special Reference to the Ultrastructural Pattern of Enamel—Robert M. Frank (Institut Dentaire Strasbourg) and Reidar F. Sognnaes (Harvard).
9. Calcifiability of the Collagenous Dentin Matrix—Clive C. Solomons, James T. Irving, and William F. Neuman (Rochester).
10. Ultrastructural Relationship Between the Organic and Inorganic Elements of Bone—Robert A. Robinson (Johns Hopkins).
11. Pathogenesis of Abnormal Tissue Calcification—Reuben Eisenstein, Richard E. Trueheart, and George M. Hass (Illinois).
12. Salivary Calculus Deposition—S. Wah Leung (Pittsburgh).
13. Experimental Induction of Osteogenesis—Melvin L. Moss (Columbia).
14. Behavior of Bone in Tissue Culture—Paul Goldhaber (Harvard).
15. Comparative Studies on Structure and Chemical Composition of Connective Tissues—Karl A. Piez (NIDR) and Jerome Gross (Harvard).
16. Nature of the Inorganic Phase in Calcified Tissues—Aaron S. Posner (NBS).
17. The Specificity of the Molecular Structure of Organic Matrices in Mineralization—Melvin J. Glimcher (MIT).

New Research Journal.—Upon the horizon there appears the probability that another publication devoted to dental research on an an international plane is in its gestation period and about to be born in the very near future. While the Editor of the *J. D. Res.* to date has not been informed directly by the Pergamon Press of London of the new publication, it has been learned indirectly that a journal is to appear in 1959 under the worthy name of *Archives of Oral Biology.*

There is always room for another good publication in this rapidly expanding field. Thus, the Editor of this JOURNAL heartily welcomes this new publication into the fold of scholarly journalism concerned with research in the oral region. *Bonis avibus.*

FRANK J. ORLAND, D.D.S., PH.D.
EDITOR

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH ANNOUNCEMENT OF 1959 GENERAL MEETING

The 37th General Meeting of the International Association for Dental Research will convene at the Sheraton-Palace, San Francisco, California, on Thursday, Friday, Saturday, and Sunday, March 19, 20, 21, and 22, 1959. The Dental Materials Group of the Association will meet on Friday, Saturday, and Sunday, March 20, 21, and 22. The American Association of Dental Schools will meet at the same place beginning on Sunday, March 22.

Notice to Prospective Program Participants.—Abstracts of items for the program must be limited to 250 words *including* title, authors' names, etc. Three copies are required. *For further instructions, contact Dr. Burrill, Secretary-Treasurer.* Nonmembers are invited to submit items for possible presentation on the program. Sponsorship by a member is requested in such cases. Abstracts of papers on the program will be published in the JOURNAL OF DENTAL RESEARCH *after* the meeting. The members have been polled for an expression of opinion on whether or not the abstracts should also be preprinted, and they voted to have no preprinted abstracts with a January first deadline.

By Council action at the 1958 meeting, the Read-by-Title section has been eliminated from the program for the 1959 meeting.

Abstracts entered in the *Edward H. Hatton* Award Competition should conform to the specifications published in the JOURNAL OF DENTAL RESEARCH 33: 590, 1954. Entries should be clearly marked as such.

DAN Y. BURRILL, SECRETARY-TREASURER
Northwestern University Dental School
311 E. Chicago Avenue
Chicago 11, Illinois

A STUDY OF THE INORGANIC CARBON DIOXIDE IN INTACT HUMAN ENAMEL

M. F. LITTLE AND FINN BRUDEVOLD

Eastman Dental Dispensary, Rochester, N. Y.

THE wide variations in the reported values for the CO_2 content of human enamel[1, 2] may reflect actual changes in the CO_2 distribution during and after mineralization.

In human material, teeth of fetuses were found to contain less CO_2 than erupted teeth[3]; hypoplastic enamel, more CO_2 than well-calcified enamel[4]; and, in one study, portions of enamel of the same teeth, different amounts of CO_2.[5] In animal experiments, Sobel showed that the CO_2 content of not only bones but also enamel and dentin can be altered by changing the CO_3:PO_4 ratio of the blood with diets of different Ca : P ratios.[6] An increase in the CO_2 content in the enamel was correlated with increased caries susceptibility.[7] However, attempts to correlate the CO_2 content in human dentin or enamel with caries[2, 8] have been inconclusive. Similarly, the effect of age on the CO_2 content of enamel has not been established.

If the CO_2 of enamel mineral, like bone,[9] can be preferentially removed by acids, then the acids formed in the mouth by carbohydrate degradation should cause detectable losses of CO_2 in at least the outer portions of human enamel. In addition, since fluoride has been reported to replace carbon dioxide in ground enamel,[10] the accumulation of fluoride with age in the surface of enamel[11] might be expected to cause a small gradual reduction in the carbon dioxide content. These considerations suggested that studies be undertaken to determine (1) if there are differences in CO_2 content of surface and subsurface enamel of unerupted and erupted teeth, (2) whether CO_2 content in the enamel changes with age, and (3) if related changes occur in the Ca, inorganic P, or ash weight of the samples.

Since recent infrared absorption studies have shown that the CO_2 in fresh enamel is present in the form of carbonate and not bicarbonate,[12] the term carbonate could be used in general discussion of the radical in this paper. However, the results are reported as per cent CO_2 dry weight unless otherwise noted.

MATERIALS AND METHODS

Extracted permanent teeth obtained by exodontists in Buffalo, N. Y., a low fluoride area, were grouped: unerupted—not fully formed (judged by an almost complete lack of roots), and fully formed; erupted—under 20, 20-29, 30-49,

This study was supported in part by U.S. Public Health Service Grant No. D-215.
Partial support was also received from Bristol Myers Co.
Read in part at the 33rd General Meeting of the International Association for Dental Research, Chicago, Ill., March, 1955.
Received for publication Oct. 4, 1957; revised by authors June 10, 1958.

and over 50 years of age. The teeth were collected on aluminum trays in bottles containing moistened cotton and a little thymol. Restorations and carious material were removed with a dental bur and the teeth were cleaned by scraping, rinsed with distilled water, and stored in a freezer.

Pooled samples of 6 to 8 successive layers of enamel were obtained from the smooth surfaces of about 60 teeth in each age group by a previously described method of grinding.[11] In most of the groups, these layers represented a total of between one sixth to one third of the enamel. In one group (under 20), layers were ground from the surface to the dentinoenamel junction. The first few layers of each group were ground lightly in order to obtain detailed information about the outermost enamel. In order to make comparisons between the groups, the thickness of the ground enamel layers had to be estimated. An approximation of the thickness was obtained after the method of Brudevold,[11] by dividing the weight of the samples by the estimated surface area of the teeth from which they were ground.

The ground samples were dried to constant weight at 105° C. Aliquots of each sample were ashed in platinum combustion boats at 525° C. in order to estimate the amount of organic material and lattice water present and to calculate carbon dioxide figures on an ash weight basis.

TABLE I

PERCENTAGE CARBONATE (CO_2) IN STANDARD SAMPLES ON DRY WEIGHT BASIS

COMPOSITION	NUMBER OF DETERMINATIONS*	THEORETICAL CO_2 CONTENT	CO_2 RECOVERED	PER CENT RECOVERY
$Na_2CO_3/NaCl$	5	1.68	1.68 ± .01†	100.0
$Na_2CO_3/NaCl$	3	1.94	1.88 ± .01	96.9
$CaCO_3/NaCl$	12	2.00	1.98 ± .02	99.0
$CaCO_3/NaCl$	13	2.06	2.04 ± .02	99.0
$CaCO_3/NaCl$	15	2.52	2.49 ± .01	98.8
NBS Ia limestone/Ca_3PO_4	7	2.64	2.61 ± .01	98.9
Pooled enamel	6	—	2.32 ± .01	—
Pooled enamel (outer portions)	14	—	1.60 ± .01	—

*Size of sample ranged from 15 to 25 mg.
†Standard deviation.

Aliquots of 15-20 ± .01 mg. of the ground enamel were weighed on a Sartorius-Werke semimicro-balance and analyzed for inorganic CO_2 content by the Sobel modification[13] of the van Slyke-Folch manometric method[14] using McFayden's reagents and factors.[15] In order to establish the method, a series of samples and a primary standard* were prepared with CO_2 concentration within the range expected for enamel. Recovery and standard deviation are shown in Table I. Pooled enamel samples were found to give a similar standard deviation. Known standards and a reagent blank were run concurrently with ground enamel samples. Three or more determinations of each sample with a maximum mean deviation of ± 0.01 were used for all reported values.

Inorganic calcium[16] and phosphorus[17] were analyzed by established procedures on aliquots of the dried samples. The precision as measured by standard deviation was within 7 parts per 1,000 for Ca and 3 parts per 1,000 for P.

*National Bureau of Standards, Argillacious limestone Ia,

RESULTS

The percentage of CO_2 in the different layers is given in Table II. Every group of teeth, whether unerupted or erupted, shows an increase in CO_2 content from the surface inward. This increase is gradual from the first to the sixth layer.

TABLE II

PERCENTAGE CARBONATE (CO_2) IN SUCCESSIVE LAYERS OF ENAMEL FROM PERMANENT TEETH OF DIFFERENT AGES, ON DRY WEIGHT BASIS

	UNERUPTED		ERUPTED			
LAYER	NOT FULLY FORMED	FULLY FORMED	UNDER 20	20-29	30-49	OVER 50
1	1.65	1.72	1.66	1.51	1.35	1.38
2	1.80	1.73	1.88	1.59	1.46	1.57
3	2.01	1.79	2.04	1.70	1.68	1.66
4	2.04	1.90	2.17	1.77	1.96	1.94
5		2.03	2.21	1.86	2.21	2.10
6		2.11	2.36	1.99	2.22	2.00

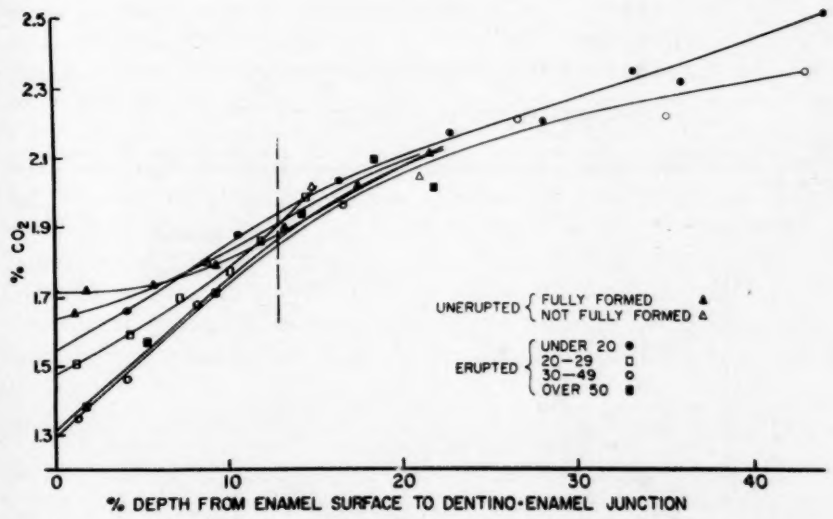


Fig. 1.—The distribution of inorganic CO_2 (as per cent dry weight) in enamel ground from the intact smooth surfaces of (a) unerupted teeth grouped according to whether the root was or was not fully formed, and (b) erupted teeth grouped according to age.

In comparing the two outer layers of each erupted group, there appears to be a decrease in CO_2 with age. The effect of age is more reliably shown in Fig. 1. Here the variable thickness of the ground layers is taken into consideration and the CO_2 content of each layer is plotted against its calculated distance from the outer surface. The distribution curves show that there is more CO_2 in the outer layers of unerupted than of erupted teeth, and that the loss in CO_2 with age is confined to approximately the outer one tenth of the enamel (marked by broken vertical line).

In Fig. 2, the decrease in CO_2 content with age in the outermost enamel is shown graphically. Before eruption there appears to be a slight increase

in the surface CO_2 as the enamel matures. After eruption this trend is reversed and there is a steady decrease up to the age of 40 to 50 years.

The CO_2 data for the under 20-year group, which included layers from the surface to the dentinoenamel junction, are plotted in Fig. 3. There is a gradual increase in the CO_2 content from the surface inward, with a maximum

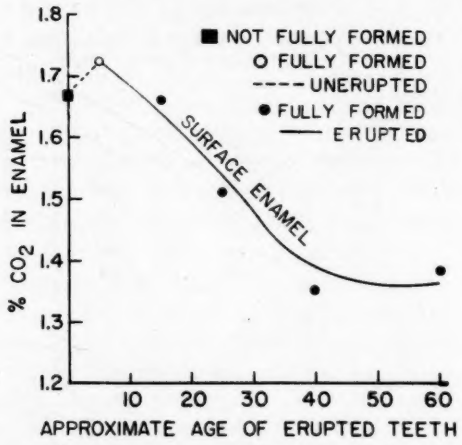


Fig. 2.—The change in the inorganic CO_2 content of the outermost layer of smooth-surface enamel, (a) before eruption with increasing root formation and (b) after eruption with increasing age.

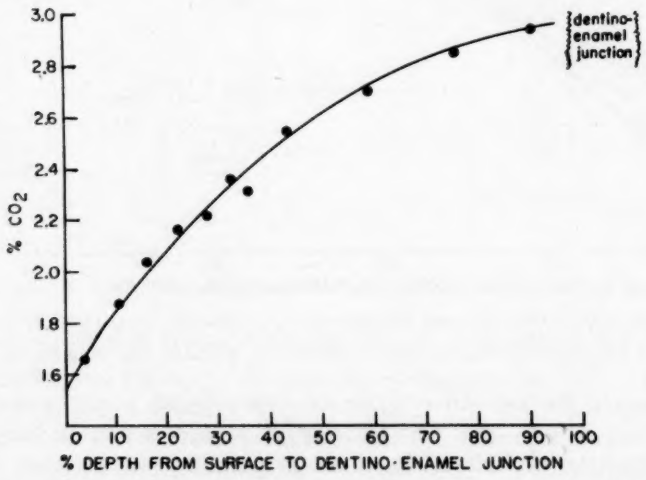


Fig. 3.—The distribution of inorganic CO_2 (as per cent dry weight) in the smooth-surface enamel of permanent teeth from persons under 20 years of age.

of almost 3.0 per cent occurring near the dentinoenamel junction. In grinding the last enamel layer, small amounts of junction material and dentin were included. After fractionation in bromoform-acetone mixtures, two portions of enamel were obtained with 99.8 per cent purity,[18] one with a density between

2.80 and 2.89, and the other with a density over 2.89. These fractions gave a carbon dioxide content of 2.96 and 2.83 per cent, respectively, suggesting that enamel from the same layer may not only have different densities but also different concentrations of CO_2.

TABLE III
PERCENTAGE LOSS OF WEIGHT IN SUCCESSIVE LAYERS OF ENAMEL ON ASHING AT 525° C.

	UNERUPTED		ERUPTED			
LAYER	NOT FULLY FORMED	FULLY FORMED	UNDER 20	20-29	30-49	OVER 50
1	3.5	3.67	3.19	3.2	4.57	6.4
2	2.7	2.85	3.69	2.7	4.28	3.61
3	2.8	2.64	2.70	1.3	3.27	3.49
4		2.39	3.26	3.7	2.29	3.03
5			3.54	3.9	2.21	2.84
6			3.9	2.8	2.02	2.62
7					2.12	

So far, the CO_2 data have been considered on a dry weight basis. Because of the higher organic content in surface than subsurface enamel,[19] CO_2 values on an ash basis were also calculated. Table III shows the loss in weight of the different layers of enamel on ashing at 525° C. In most of the groups, a slightly larger amount of material, assumed to be organic matter and water,[20] was lost from the two first layers than from deeper enamel layers. It can also be seen that the weight lost from the outer enamel on ashing tends to increase with age. The two impacted groups show a similar loss in weight on ashing and thus would have similar degrees of mineralization,[20] despite the obviously earlier formative stage of the tooth in the group with not fully formed roots. Ashings were also carried out at 900° C. The additional loss in weight between 525° and 900° C. approximated the amount of CO_2 found by chemical analysis. The ashed residue retained less than 0.11 per cent CO_2 after 30 minutes at 900° C.

TABLE IV
PERCENTAGE CARBONATE (CO_3) IN SUCCESSIVE LAYERS OF ENAMEL CALCULATED ON ASH WEIGHT BASIS (525° C.)

	UNERUPTED		ERUPTED			
LAYER	NOT FULLY FORMED	FULLY FORMED	UNDER 20	20-29	30-49	OVER 50
1	1.70	1.79	1.71	1.56	1.41	1.47
2	1.85	1.78	1.95	1.63	1.52	1.63
3	2.07	1.84	2.10	1.72	1.74	1.75
4		1.95	2.24	1.84	2.01	2.00
5			2.29	1.94	2.26	2.16
6			2.52	2.46	2.27	2.05

Table IV gives the CO_2 values calculated as percentage of the 525° C. ash weight. The distribution of CO_2 appears to be similar to the CO_2 results calculated on dry weight basis. Thus, the differences noted between the CO_2 content of the surface and subsurface enamel are not accounted for by equating to an organic free basis, but must represent real differences in the concentrations of CO_2 in the mineral phase of the enamel.

TABLE V

PER CENT INORGANIC CALCIUM AND PHOSPHORUS IN SUCCESSIVE LAYERS OF ENAMEL FROM PERMANENT TEETH OF DIFFERENT AGES, ON DRY WEIGHT BASIS

	UNERUPTED				ERUPTED											
	NOT FULLY FORMED	FULLY FORMED			UNDER 20			20-29			30-49			OVER 50		
LAYER	P	Ca	P	Ca : P	Ca	P	Ca : P	Ca	P	Ca : P	Ca	P	Ca : P	Ca	P	Ca : P
1	17.5	36.7	17.7	2.07	35.0	18.0	1.94	—	17.6	—	38.9	17.3	2.25	35.8	17.7	2.02
2	17.5	37.0	17.6	2.10	36.4	17.7	2.06	—	17.8	—	38.0	17.8	2.14	37.8	17.7	2.14
3	17.6	—	17.7	—	37.5	17.8	2.11	—	17.8	—	38.8	17.9	2.17	36.4	17.6	2.07
4	17.8	—	17.9	—	37.8	18.0	2.11	—	17.7	—	36.2	17.7	2.04	36.2	17.7	2.04
5		—	17.6	—	37.6	17.9	2.10	—	17.5	—	35.7	17.6	2.03	37.0	17.7	2.09
6		—	17.4	—	37.6	17.8	2.11	—	17.6	—	36.7	17.6	2.08	37.4	17.8	2.10
7					—	17.7	—				36.7	17.4	2.08			

Despite a slight variation in the calcium and inorganic phosphorus content of the mineral phase of the enamel, Table V shows that no marked differences were found with age or enamel depth. It can be seen that the Ca:P weight ratio hovers around 2.08 (molar ratio 1.57). This is in marked contrast to the molar ratios CO_2:P shown in Table VI. Here, a consistent increase in the ratio of CO_2:P is seen from the surface inward in every group, with a high of 0.118 occurring in the enamel close to the dentinoenamel junction. There is an obvious decrease in the CO_2:P ratio in the outermost enamel after eruption with increasing age.

TABLE VI

MOLAR CO_2: P RATIO IN SUCCESSIVE LAYERS OF ENAMEL ON DRY WEIGHT BASIS

	UNERUPTED		ERUPTED			
LAYER	NOT FULLY FORMED	FULLY FORMED	UNDER 20	20-29	30-49	OVER 50
1	.066	.068	.065	.060	.055	.055
2	.072	.069	.075	.063	.058	.062
3	.080	.071	.081	.067	.066	.068
4	.081	.075	.085	.070	.078	.077
5		.081	.087	.075	.088	.084
6		.085	.093	.080	.089	.079
11			.118			

DISCUSSION

The present findings show a decrease in the concentration of CO_2 in the mineral phase of the enamel from the dentinoenamel junction toward the enamel surface in all tooth groups. The fact that the decrease occurs even in the incompletely formed unerupted teeth suggests that this pattern of distribution developed at the time of enamel calcification or shortly thereafter.

Engfeldt and Hammarlund Essler[21] and others[22] have shown that enamel calcification proceeds from the dentinoenamel junction toward the outer enamel; this study shows that less CO_2 is also incorporated in the enamel from the dentinoenamel junction toward the enamel surface. A similar observation has been made on bone. Here the initial solid formed is lower in CO_2 than the same solid at a later stage, i.e., "new" bone is lower in CO_2 than "old" bone.[23] When enamel is fully calcified, the inner enamel is the "oldest" calcified material of the enamel and like old bone it has the highest content of CO_2. A slightly lower concentration of carbon in the surface enamel of incompletely formed unerupted teeth indicates that a build-up of carbon dioxide may occur after mineralization.

The mechanism which brings about these differences in enamel has not been established, although a logical explanation can be deduced from prior work on mineral calcification.

The differential in CO_2 content between the surface and dentinoenamel junction may indicate a difference in the fluids of the enamel organ during calcification since the CO_2:P ratio of calcified structures has been shown to reflect the CO_2:P in the blood[6] and most likely the fluids[9, 24] from which they are formed. This would suggest that the tissue fluids in the enamel organ

change as calcification proceeds. In in vitro studies, Strates[25] observed that an increase in acidity accompanies increased hydroxyapatite formation. If a similar reaction occurs in vivo, the levels of [HCO_3^-] in the fluid at the calcification front should decrease with increased deposition of hydroxyapatite, and the new apatite formed would have progressively less CO_2 incorporation.[24]

Since a slightly higher concentration of CO_2 was found in the outer enamel of fully formed than that of incompletely formed unerupted teeth, the tissue and blood CO_2 levels are probably higher at this stage than during active calcification.[24] The same appears to be true of bone. The blood CO_2:P increases with decreasing calcification and the bone salts increase in CO_2 apparently by equilibration.[24]

After tooth eruption, there is a gradual loss of the CO_2 fraction with age. This posteruptive loss may be related to the chemical environment of the mouth. Experiments not reported in this study have shown that part of the CO_2 in ground enamel can be preferentially removed. For example, a small fraction can be extracted by washing in distilled water, and up to approximately 20 per cent by exposure to weak acid. In this respect enamel appears to behave much like bone.[9] Since similar amounts of CO_2 are lost by surface enamel with age, this suggests that the acid present in foods or formed from carbohydrate degradation may be a causative factor. A small amount of carbonate may also be lost by exchange with fluoride,[16] but the extent of this exchange in surface enamel is unknown.

However, it is interesting to note certain implications inherent in these findings of differences in the CO_2 from various portions of enamel. Sobel has shown that enamel with high CO_2:P ratios lost carbon dioxide more readily in acid solutions than enamel with a low CO_2:P ratio.[26] The enamel cited by Sobel had molar ratios of 0.128 and 0.065, respectively. Outermost enamel is reported in this study to have a CO_2:P ratio around 0.06; and the enamel closest to the dentinoenamel junction, 0.118. Studies in this laboratory[27] have shown that the outermost enamel is also less soluble in acid than inner enamel. This suggests that the formation of surface enamel with low CO_2:P ratios may be a fortuitous protective device, since teeth with low CO_3:PO_4 have been found to be more caries resistant than enamel with higher ratios.[26] Similarly, the slow preferential posteruptive loss of CO_2 from the surface of enamel may increase its resistance to acid attack and further contribute to the caries resistance of the teeth.

SUMMARY

1. Differences in the CO_2 content of superficial and inner portions of intact human enamel are reported for unerupted and erupted permanent teeth of different ages. The concentration of CO_2 increases from the surface toward the dentin in all tooth groups. This pattern of distribution appears to be developmental. The CO_2 content of the outermost and subsurface enamel increases after the completion of calcification in unerupted teeth, and decreases progressively with age in erupted teeth. The CO_2 distribution in the bulk of the enamel does not change after mineralization.

2. The loss in weight on ashing at 525° C. is greater in the outermost than in immediately underlying enamel. However, the pattern of distribution of CO_2, based on either dry or ashed weight, is similar.

3. No marked differences were found in the calcium or inorganic phosphorus distribution with age or enamel depth. CO_2:P molar ratio ranged from a low of 0.05 at the outer surface of enamel to a high of 0.115 near the dentinoenamel junction.

4. Preliminary experiments show that a portion of the CO_2 in enamel may be removed by exposure to weak acids. Loss of CO_2 from the surface enamel of erupted teeth may therefore be the result of preferential dissolution.

The authors wish to acknowledge the technical assistance of J. Rowley and R. Turner and the special cooperation of Drs. B. G. Wakefield, S. C. Waite, M. D. Wolfson, and C. Lange of Buffalo, New York, in obtaining tooth material.

REFERENCES

1. Leicester, H. M.: Biochemistry of the Teeth, St. Louis, 1949, The C. V. Mosby Company.
2. French, E. L., Welch, E. A., Simmons, E. S., LeFevre, M. L., and Hodge, H. C.: Calcium, Phosphorus and Carbon Dioxide Determination of All the Dentin From Sound and Carious Teeth, *J. D. Res.* **16**: 401, 1938.
3. LeFevre, M. L., Bale, W. F., and Hodge, H. C.: Chemical Nature of the Inorganic Portion of Fetal Tooth Substance, *J. D. Res.* **16**: 85, 1937.
4. Bowes, J. H., and Murray, M. M.: The Chemical Composition of Teeth. III. The Variation in Chemical Composition in Relation to Dental Structures, *Biochem. J.* **30**: 977, 1936.
5. Bremer, G.: Untersuchungsmethodik und vergleichenge chemische Analysen on Zähnen von Homo Affe, Hund und Schwein, *Upsala läkaref förh.* **44**: 219, 1939.
6. Sobel, A. E., and Hanok, A.: Calcification of Teeth. I. Composition in Relation to Blood and Diet, *J. Biol. Chem.* **176**: 1103, 1948.
7. Sobel, A. L., Hanok, H., and Shaw, J. H.: Abstracts 124th Meeting, American Chemical Society, Abst. No. 173, p. 72, 1953.
8. Armstrong, W. D., and Breckus, P. J.: Chemical Constitution of Enamel and Dentin. I, *J. Biol. Chem.* **120**: 677, 1937.
9. Logan, M. A., and Taylor, H. L.: Solubility of Bone Salts. III. Partial Solution of Bone and Carbonate Containing Calcium Phosphate Precipitates, *J. Biol. Chem.* **125**: 391, 1938.
10. McCann, H. G., and Bullock, F. A.: Reaction of Fluoride Ion With Powdered Enamel and Dentin, *J. D. Res.* **34**: 59, 1955.
11. Brudevold, F., Gardner, D. E., and Smith, F. A.: The Distribution of Fluoride in Human Enamel, *J. D. Res.* **35**: 420, 1956.
12. Underwood, A. L., Toribara, T. Y., and Neuman, W. F.: An Infrared Study of the Nature of Bone Carbonate, *J. Am. Chem. Soc.* **77**: 317, 1955.
13. Sobel, A., Rockenmacher, M., and Kramer, B.: A Microestimation of the Inorganic Constituents of Bone, *J. Biol. Chem.* **152**: 255, 1944.
14. Van Slyke, D. E., and Folch, J.: Microestimation of Organic Carbon by Wet Combustion, *J. Biol. Chem.* **136**: 509, 1940.
15. McFayden, D.: Determination of Amino Acids in Plasma by the Van Slyke Method, *J. Biol. Chem.* **145**: 387, 1942.
16. Chen, R. S., and Toribara, T. Y.: Determination of Calcium in Biological Materials by Flame Photometry. Atomic Energy Report No. 247, University of Rochester, Rochester, New York.
17. Willard, H. H., and Center, E. J.: Colorimetric Determination of Phosphorus in Iron Ore, *J. Indust. Eng. Chem. Anal. Ed.* **13**: 81, 1941.
18. Manly, R., and Hodge, H. C.: Density and Refractive Index Studies of Dental Hard Tissues. I. Methods for Separation and Determination of Purity, *J. D. Res.* **18**: 133, 1939.
19. Coolidge, T. B.: Nitrogen and Phosphorus of Early, Smooth-Surface Caries of Enamel, *J. D. Res.* **30**: 97, 1951.
20. Deakins, M. J.: Changes in the Ash, Water, and Organic Content of Pig Enamel During Calcification, *J. D. Res.* **21**: 429, 1942.

21. Engfeldt, B., and Hammarlund Essler, E.: Studies on Mineralized Dental Tissues. IX. A Microradiographic Study of the Mineralization of Developing Enamel, *Acta odont. scandinav.* **14**: 273, 1956.
22. Belanger, L. F.: The Mineralization of Rat Enamel in the Light of Ca^{45} Autoradiography and Microincineration, *J. D. Res.* **36**: 595, 1957.
23. Kramer, B., and Shear, M. J.: Composition of Bone. IV. Primary Calcification, *J. Biol. Chem.* **79**: 147, 1928.
24. Neuman, W. F., and Neuman, M. W.: Nature of the Mineral Phase of Bone, *Chem. Rev.* **53**: 28, 1953.
25. Strates, B. S.: The Mechanism of Calcification. Thesis, University of Rochester, Rochester, N. Y., 1956.
26. Sobel, A. E.: Local Factors in the Mechanism of Calcification, *Ann. New York Acad. Sc.* **60**: 719, 1955.
27. Isaac, S., Brudevold, F., Smith, F. A., and Gardner, D. E.: Solubility Rate and Natural Fluoride Content of Surface and Subsurface Enamel, *J. D. Res.* **37**: 255, 1958.

FURTHER STUDIES ON THE ACTION OF COLLAGENASE ON SOUND AND CARIOUS HUMAN DENTIN

W. G. ARMSTRONG

Institute of Dental Surgery, University of London, Eastman Dental Hospital, London, W.C. 1, England

INTRODUCTION

EARLIER work in this field by Evans and Prophet[1, 2] showed that decalcified human dentin was disintegrated by the extracellular collagenase enzymes elaborated in broth cultures of strains of *Clostridium welchii* and *Cl. histolyticum*. Fully calcified dentin was found to be resistant to the enzymes, and a subsequent investigation by Atkinson and Prophet[3] showed that decalcified carious human dentin was also apparently resistant to their collagenase preparations. They concluded that during the development of caries of the dentin the organic matrix had been modified in some way to a collagenase resistant form.

Konetzka, Burnett, and Pelczar[4] extended these studies using sound dentin decalcified, at neutral pH, by the chelating agent ethylenediaminetetraacetic acid (EDTA). They demonstrated that EDTA-decalcified dentin was apparently resistant to their collagenase preparations, whereas acid-decalcified dentin, which was used in the experiments of Prophet and his coworkers, was attacked by the enzyme. On the basis of their results, Konetzka and associates[4] suggested that the collagenase resistance of carious dentin might be interpreted as evidence that the demineralization process in dentin caries involved chelating mechanisms.

The present paper reports on observations made in further studies into the action of collagenase on sound dentin, and upon the nature of the collagenase resistance found in carious dentin. A subsequent investigation[5] shows the effect of chemical modification of specific groups present in dentin collagen upon its susceptibility to collagenase attack.

MATERIALS AND METHODS

Preparation of Dentin.—

Sound dentin: Freshly extracted teeth were collected daily, well washed, split open longitudinally, and dried in a vacuum desiccator over phosphorus pentoxide. The cementum was removed by grinding the teeth against a slowly revolving, continually moistened, carborundum wheel. The crowns and apices were clipped off, and the pulp and inner aspect of the dentin removed with a

Received for publication Dec. 12, 1957; revised by author June 24, 1958.

dental burr. The dentin was then pulverized in a steel mortar, and the particles screened through standard mesh sieves. Only particles passing a 120-mesh sieve (BS 410/1943) were used in the experiments. The dentin preparations were then cleared of contaminating steel particles magnetically. Further purification by flotation procedures[6] indicated negligible contamination by particles of different density to the dentin. The dentin samples were grouped as sound dentin prepared from sound teeth, and sound dentin prepared from carious teeth. A subsequent investigation showed that there was no detectable difference in susceptibility to attack by collagenase between sound dentin prepared from carious teeth and that prepared from sound teeth. Carious teeth, which were more readily obtainable, were therefore used for the preparation of sound dentin.

Carious dentin: Carious dentin of a leathery consistency was removed from freshly extracted carious teeth with a dental excavator, then dried, pulverized, and screened as for sound dentin.

Decalcified dentin: Dentin samples prepared as described above were decalcified overnight (circa 18 hours) at room temperature, either by stirring with M/5 HCl or with a 20 per cent solution of ethylenediamine-tetraacetic acid (EDTA) buffered at pH 7.4. The amount of decalcifying agent added was in 50 per cent excess of that theoretically required for complete demineralization. The decalcified dentin was washed 5 times with distilled water, 3 times with acetone, and then air dried at room temperature. Ash values of the decalcified preparations were determined by slowly incinerating weighed amounts, at low temperatures, in a crucible, and then heating the residues for 2 hours at 550° C. in an electric muffle furnace. Samples of dentin demineralized by the methods described gave ash values of 1 to 2 per cent.

Collagenase Preparations.—

1. *Broth culture filtrate precipitates:* The following organisms, from the National Collection of Type Cultures, were used for the production of crude enzyme preparations: *Cl. welchii* S 107 (Cat. No. 8327), *Cl. welchii* S.R.12 (Cat. No. 6784), and *Cl. histolyticum* CN 920 (Cat. No. 7124). Small quantities of enzyme preparation were obtained from 24-hour cultures grown on Robertson's meat broth medium in McCartney bottles. After centrifuging, the supernatant was collected, and ammonium sulfate added to saturation point. The light precipitate which eventually floated to the surface was collected and dissolved in 1 ml. phosphate buffer pH 7.4.

2. *Partially purified collagenase preparation:* The Wellcome Research Laboratories supplied a partially purified freeze-dried collagenase preparation (B 637 C). This contained the following: collagenase (κ-toxin), 22 Q units/mg.*; α-toxin, 4.3 units/mg.; μ-toxin, 8 units/mg.; protein nitrogen, 0.036 mg./mg.

3. *Antisera:* These were also supplied by the Wellcome Research Laboratories. A list of the antitoxins present in each follows.

*The Q unit is an arbitrary measure of collagenase activity and is defined as the amount of enzyme which, under defined conditions, will liberate a standard quantity of soluble dyestuff from an azocoll preparation.[12]

Antitoxin in antiserum	Antiserum RX 5755/36 (U./ml.)	Antiserum R 1974 (U./ml.)
anti α	220	45
anti β	50,000	0
anti ϵ	2,500	0
anti θ	90	0
anti μ	670	5
anti κ	1	400
anti λ	6,000	4

Detection and Estimation of Collagenase Action on Dentin.—

a. *Agar plate method:* The method developed by Evans and Prophet[1] was used; the 4 per cent agar was made up by dissolving Bacto-agar in M/15 Sorensen phosphate buffer, pH 7.4.

b. *Assay of amino group N liberated:* Approximately 10 mg. of each dentin sample were weighed into a stoppered 6 by 1 inch Pyrex boiling tube. Phosphate buffer pH 7.4, 0.5 ml., was added, and each boiling tube was placed in a thermostatically controlled water bath at 37° C. A 30 mg. per cent collagenase solution was prepared by dissolving a weighed quantity of the B 637 C collagenase preparation in buffer. Following equilibration at 37° C., a 3 ml. aliquot of the enzyme solution was added to each boiling tube. The tubes were slowly shaken in the water bath. At suitable time intervals 0.2 ml. aliquots of the supernatant were removed, and pipetted into centrifuge tubes containing 1 ml. absolute alcohol. After mixing and standing for not less than 15 minutes, the centrifuge tubes were centrifuged, and 1 ml. of the supernatant removed to 10 ml. graduated test tubes. The free α-NH_2 N was then estimated using the modified ninhydrin procedure described by Yemm and Cocking.[7] Control tubes with dentin and buffer only, and enzyme and buffer only, were also set up.

Micro-Kjeldahl Estimations.—

The samples were digested on an electrically heated apparatus using the copper-selenium catalyst described by Tristram.[8] The ammonia liberation, and distillation into boric acid solution, was carried out using a Markham still.[9] The ammonia was estimated by titration with M/50 or M/100 HCl using a bromcresol green/methyl red indicator.[10]

EXPERIMENTAL AND RESULTS

1. *Agar Plate Experiments.—*

a. *Preliminary investigation:* The experiments of Evans and Prophet[1] and Prophet and Atkinson[3] were repeated using acid-decalcified dentin particles. Broth culture filtrates and enzyme preparations were obtained from 3 strains of Clostridia used by them. Their work was substantially confirmed. The purified collagenase preparation B 637 C gave identical results. Although no clear zone developed around the enzyme wells in agar plates containing decalcified carious dentin particles, a barely perceptible zone of slightly altered appearance was often observed. The appearance suggested a possible action of collagenase on the carious dentin particles not readily detectable using

the agar plate technic. Control agar plates, without added dentin particles, showed that the effect observed was not due to reaction of the enzyme preparations with some component present in the agar gel.

b. *Effect of decalcification procedure upon susceptibility to collagenase attack:* Konetzka, Burnett, and Pelczar[4] reported evidence that sound dentin demineralized by EDTA was resistant to collagenase. This claim was investigated using enzyme preparations made from broth culture filtrates of the 3 strains of Clostridia listed. Dentin particles were decalcified by EDTA and M/5 HCl as described above. In no case was the EDTA-decalcified dentin found to be resistant to the enzyme preparations. However, our decalcification procedures differed in certain respects from those employed by Konetzka and his associates. They used 30 per cent EDTA and the demineralization was effected by 3 successive treatments extending over 3 days. For acidic decalcification they used a formic acid/sodium citrate solution, pH 3, and the procedure involved 5 successive treatments extending over 5 days. All their experiments were conducted at 37° C. We therefore repeated the demineralization procedures of Konetzka and co-workers, and then investigated the susceptibility of the decalcified dentin samples to disintegration by our enzyme preparations. Again, EDTA-decalcified dentin was not found to be resistant to collagenases produced by the strains of Clostridia employed by us.

2. *Estimation of Amino Group Liberation.*—

The agar plate studies had indicated the possibility of some reaction of collagenase preparations upon carious dentin which was not readily detectable using that particular technic. Also, from preliminary investigations into the relative susceptibilities of various modified dentin preparations, it was apparent that the agar plate method could not be utilized for quantitative comparative studies. A quantitative method was therefore developed for the detection and estimation of the breakdown of dentin by collagenase, based upon the measurement of the rate of α-NH_2 group liberation consequent upon the action of the enzyme on the organic matrix of the dentin.

The action of the collagenase preparation B 637 C on decalcified sound dentin, decalcified carious dentin, and undecalcified sound dentin was studied using this method. The results are shown in Fig. 1, where it can be seen that the decalcified sound dentin is broken down at the greatest rate. Conversely, calcified sound dentin is resistant to the collagenase; the small amount of breakdown, evident in the early stages only, has been shown to be due to some soluble component which can mostly be removed by a thorough preliminary washing of the particles. The decalcified carious dentin is clearly shown to be attacked, but at a slower rate than the decalcified sound dentin. The method was also used to compare the relative susceptibilities to collagenase attack of EDTA- and HCl-decalcified dentin samples. The results (Fig. 2) show that both decalcified preparations are attacked at approximately the same rate, confirming the earlier agar plate studies on the susceptibility of EDTA-decalcified sound dentin to collagenase attack.

3. *Comparison of Weight Loss Rate of Decalcified Matrix With Rate of Amino Group Liberation.*—

A series of tubes was set up, each containing approximately equal weighed amounts (10 mg.) of decalcified sound dentin and 0.5 ml. of phosphate buffer, pH 7.4. A second series containing decalcified carious dentin was also set up.

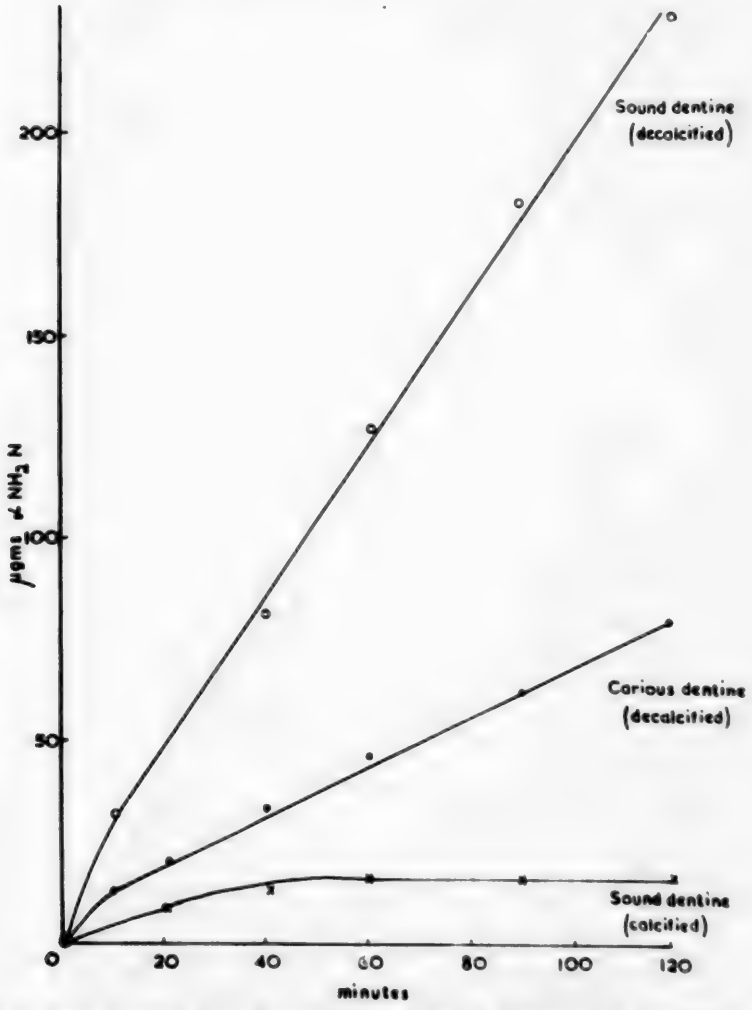


Fig. 1.—Rates of amino group liberation from action of collagenase on decalcified sound dentin, decalcified carious dentin, and calcified sound dentin.

To each tube was added 1 ml. of a 30 mg. per cent collagenase solution. The whole experiment was carried out at 37° C. At suitable time intervals, tubes were removed and the supernatants assayed for free α-NH_2 N. At the same time the residual dentin was washed, dried, and finally weighed. This weight

was then expressed as a percentage of the original amount weighed out. The α-NH_2 N released and the percentage weight values were then plotted against time as shown in Fig. 3. The results obtained indicate that the rate of α-NH_2 N group liberation approximately parallels the dentin weight loss both for sound and carious dentin. The ratio of the rates of α-NH_2 N group liberation for the sound and carious dentin agrees to within 7 per cent with the corresponding ratio of percentage weight losses, thus supporting the view that

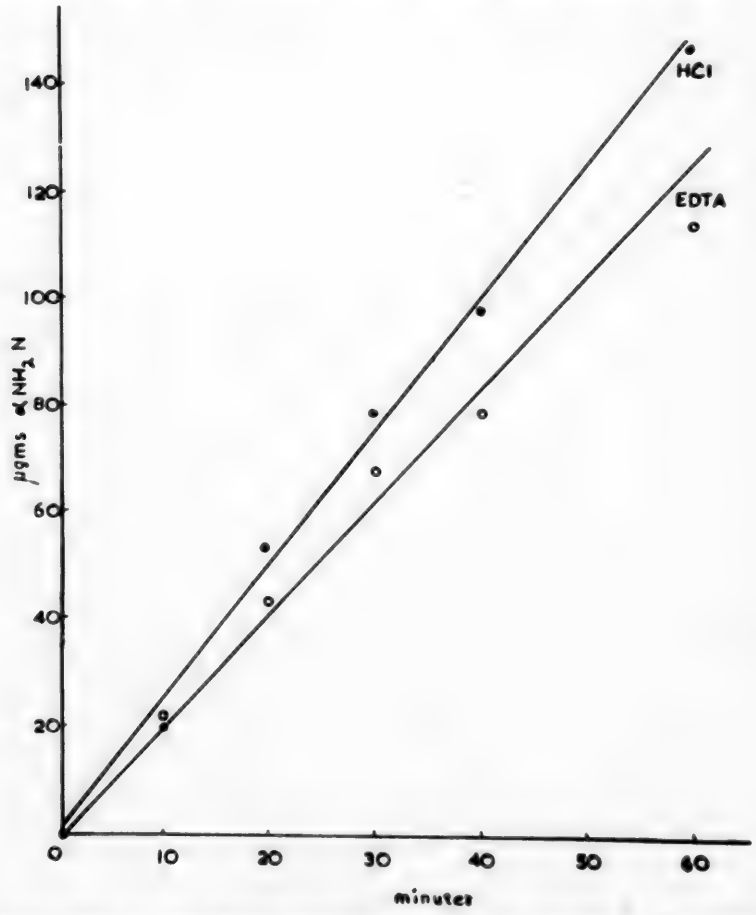


Fig. 2.—Rates of amino group liberation from action of collagenase on HCl-decalcified sound dentin and EDTA-decalcified sound dentin.

α-NH_2 N estimation provides a reliable procedure for estimating dentin breakdown by collagenase preparations. The results also show that whereas approximately 80 per cent of the decalcified sound dentin is broken down during the experimental period, only about 45 per cent of the decalcified carious dentin is attacked in the same period.

4. *Estimation of Total N Present in α-NH_2 N Fraction Assayed.*—

Examination of the experimental procedure shows that the α-NH_2 N assayed is contained in the fraction soluble in 80 per cent ethanol. Other possible products of collagenase action would be contained in the precipitate insoluble in 80 per cent ethanol, or possibly absorbed on to the residual organic matrix. Micro-Kjeldahl estimations of total N present in each fraction

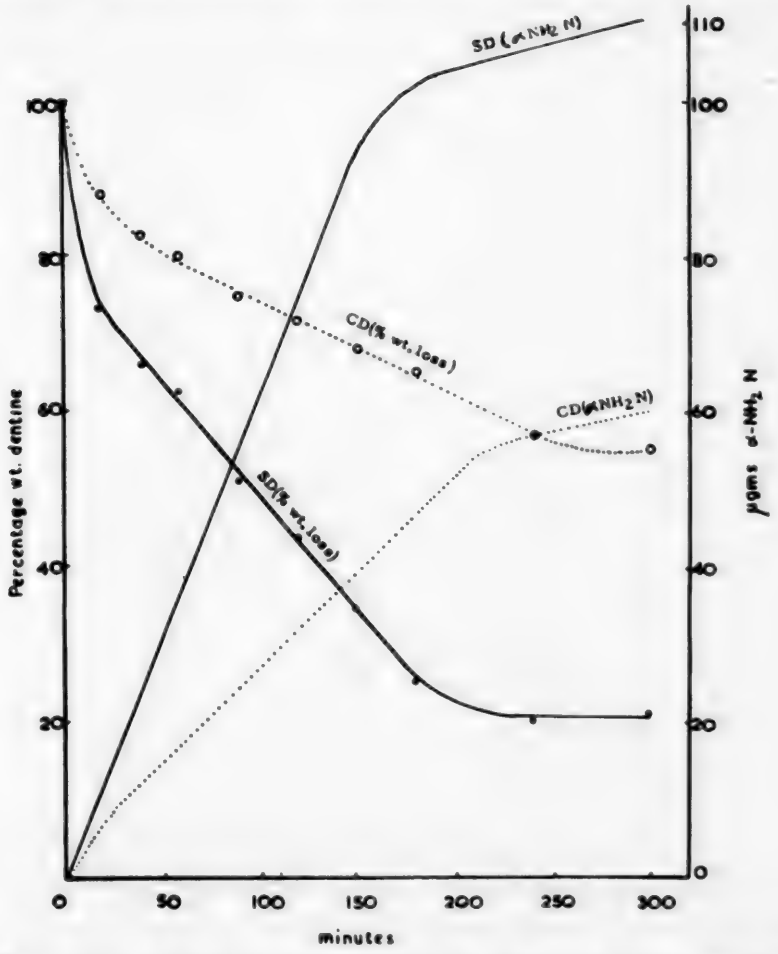


Fig. 3.—Action of collagenase on decalcified sound and carious dentin: comparison of rates of percentage weight loss with rates of amino group liberation. (*SD*: sound dentin. *CD*: carious dentin.)

were made at different time intervals to determine the relative distribution of nitrogenous products released through the action of collagenase on decalcified sound dentin.

A series of tubes was set up in a water bath at 37° C., each with a weighed amount (circa 5 mg.) of decalcified sound dentin particles and 0.5

ml. of phosphate buffer, pH 7.4. To each tube was added 1 ml. of 30 mg. per cent enzyme solution. At suitable time intervals, a tube was taken from the bath and 1 ml. of the supernatant removed to a centrifuge tube containing 4 ml. absolute alcohol. At the same time the residual dentin was washed free of enzyme with water, then washed 3 times with acetone, and finally dried in air. The 80 per cent ethanol suspension was centrifuged, and the supernatant assayed in duplicate for α-NH_2 N and total N. The alcohol-insoluble precipitate was taken up in 1 ml. of water, and a 0.5 ml. aliquot assayed for total N. Total N estimations were also made on the residual dentin fractions.

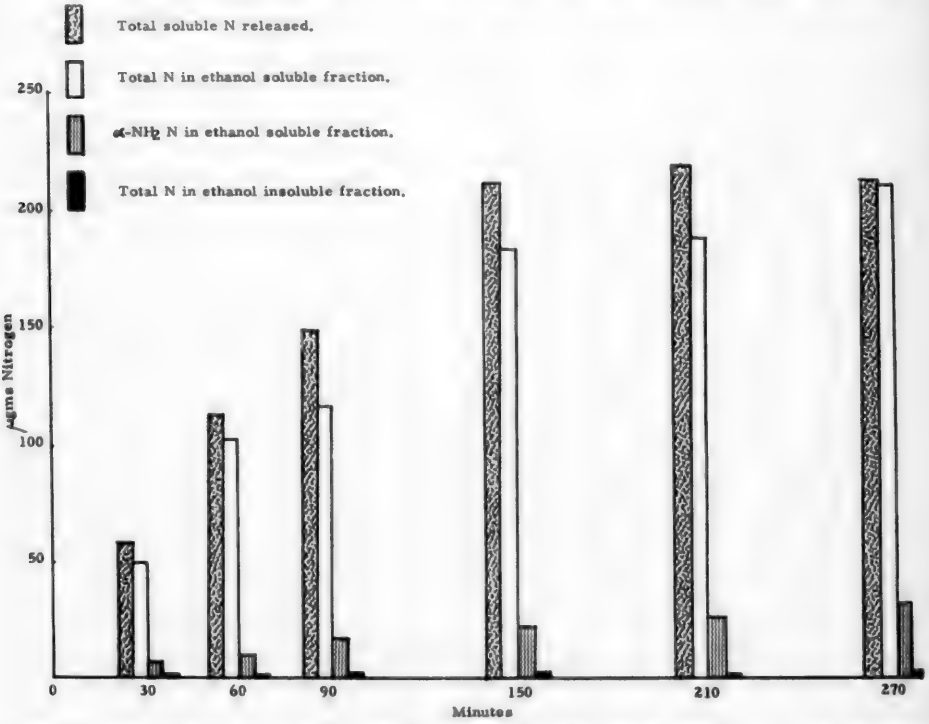


Fig. 4.—Relative distribution of soluble nitrogenous components released through the action of collagenase on decalcified sound dentin.

The results are shown in Fig. 4. The total soluble nitrogen released by collagenase action in each tube was calculated as the difference between the total N of the dentin sample weighed out and the total N of the residue remaining. It can be seen that, in this particular experiment, maximal release of soluble nitrogenous material was attained after about 3 hours. The N determinations on the 80 per cent alcohol-soluble fractions clearly show that in each tube 80 to 90 per cent of the soluble N formed was contained in this fraction. Between 10 and 14 per cent of this soluble N was in the form of free α-NH_2 N. A relatively small amount (1 to 2 per cent) of the nitrogen released through collagenase action was contained in the 80 per cent ethanol-insoluble precipitate.

5. *Paper Chromatographic Separation of the Soluble Nitrogenous Products of Collagenase Action on Dentin.*—

The previous experiment showed that only about 12 per cent of the total soluble N released through collagenase action on dentin was detectable as α-NH_2 N. This could be interpreted as indicating the release of peptide fragments from the collagen molecule, and further evidence for this was sought by means of paper chromatography.

Concentrated extracts of the 80 per cent ethanol soluble fraction were applied to strips of Whatman's No. 4 chromatography paper. A phenol-water mixture[11] was used as the developing solvent for the preparation of unidimensional chromatograms by the descending method. The separated products were revealed by spraying the dried, ether-washed strips with a 0.25 per cent solution of ninhydrin in acetone, and heating at 80° C. for 15 minutes. Six ninhydrin positive components were found in the soluble N fraction obtained from sound dentin. The same components were also found in the soluble N fraction obtained from the action of collagenase on carious dentin.

In a further experiment, larger amounts of the components were separated, and then eluted from the chromatogram. Each component was heated with 5.7 N HCl for 12 hours, and subsequent chromatography of the products showed that each component had released constituent amino acids when hydrolyzed by acid.

The paper chromatography studies clearly confirmed that the action of collagenase upon the organic matrix of dentin involves the release of a series of peptides. More detailed investigations into the structure of these products will form the basis of a separate study.

6. *Identification of Collagenolytic Factor as κ-Toxin.*—

The two specific antisera RX 5755/36 and R 1974 were used in experiments designed to establish which factors were responsible for the attack upon the organic dentin matrix. RX 5755/36 contains high titers of antibodies against a range of antigens that might be present in small quantities as contaminating factors in the B 637 C collagenase preparation; it contains almost no anti-κ antitoxin. Conxersely, R 1974 contains a high titer of anti-κ antitoxin and negligible amounts of other antibodies. A series of solutions of equivalent collagenase activity (15 Q units/ml.) were mixed with various dilutions of R 1974, and allowed to stand for 30 minutes. Two milliliters of the resultant solutions were then assayed for collagenase activity by estimation of the rate of α-NH_2 release from 10 mg. samples of EDTA-decalcified sound dentin.

In Fig. 5, the rates of α-NH_2 N release obtained have been plotted against the number of anti-κ units per Q unit of collagenase initially added to each collagenase solution. The results show that it was possible to obtain complete inhibition of collagenase action, a minimum of 5 anti-κ units per Q unit of collagenase being necessary to achieve this maximal effect.

In a similar experiment, the antiserum RX 5755/36, either alone or in the presence of submaximal concentrations of R 1974, had no detectable effect upon the action of collagenase on dentin.

These results show that the collagenase (κ-toxin) is the active factor present in the B 637 C preparation that is responsible for the attack upon the organic dentin matrix, and consequent liberation of α-NH_2 N.

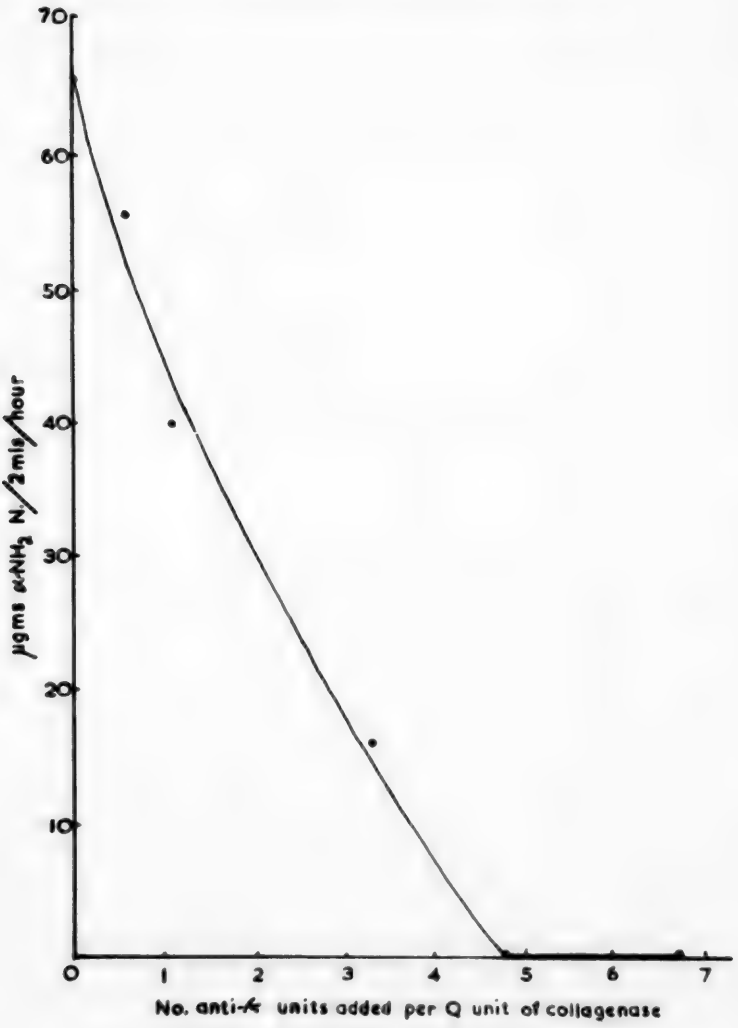


Fig. 5.—Inhibition of collagenase activity by anti-κ antibody.

7. *Examination for Presence of Collagenase Inhibitor in Carious Dentin-Collagenase Incubates.*—

The lower rate of attack by collagenase on decalcified carious dentin samples may be due to a modification of the organic matrix to a form intrinsically more resistant to enzyme attack, which occurred during the development of caries of the dentin. However, it is also conceivable that the

observed reduction in the rate of enzyme attack could be the result of an initial liberation of some collagenase inhibitory substance that had been bound on to the organic matrix during the carious process.

To test this latter possibility, the following experiment was carried out. Two tubes (I and II), the first containing 10 mg. of EDTA-decalcified dentin,

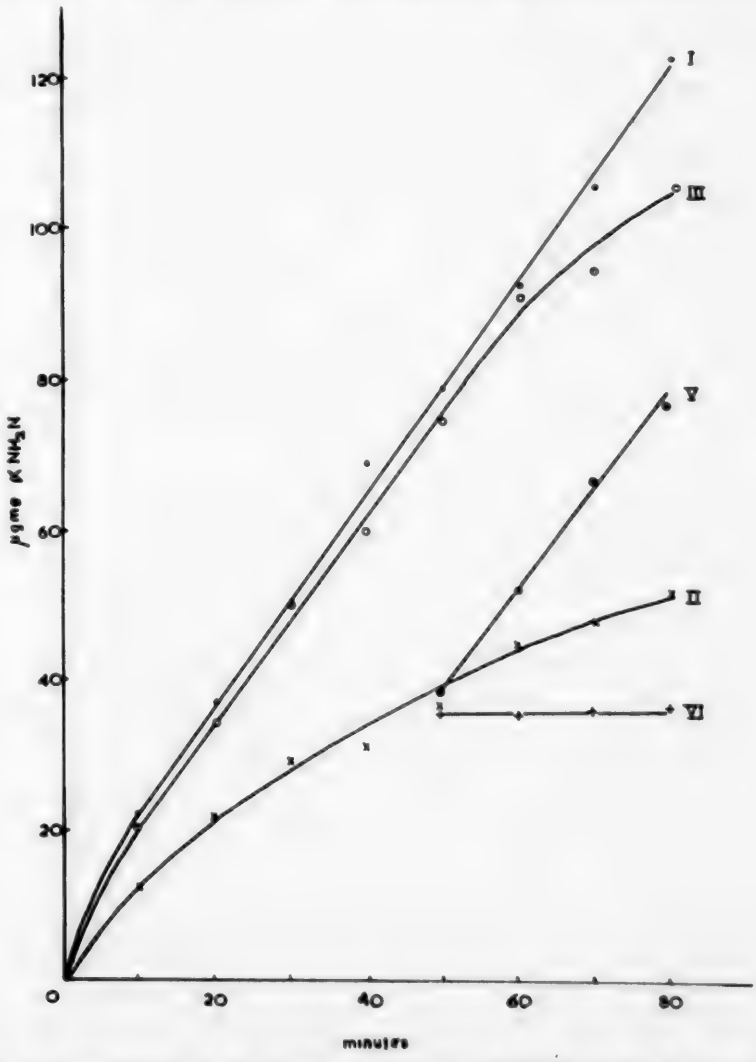


Fig. 6.—Demonstration of the absence of collagenase inhibiting factors in carious dentin-collagenase incubates.

and the second containing 10 mg. of EDTA-decalcified carious dentin, were each incubated with shaking at 37° C. with 3 ml. of a 30 mg. per cent solution of B 637 C collagenase preparation. From each tube, 0.2 ml. aliquots were removed at 10-minute intervals and assayed for α-NH_2 N in the usual way. A

third tube (III) containing 10 mg. of decalcified carious dentin, 5 mg. decalcified sound dentin, and 3 ml. of enzyme solution was similarly assayed for α-NH_2 N liberation.

At the same time, an additional tube (IV) was set up with 20 mg. of decalcified carious dentin and 6 ml. of enzyme solution, and incubated with shaking at 37° C.; no α-NH_2 N assay was made on this incubate initially, but after 40 minutes the tube was rapidly centrifuged and 3 ml. of the supernatant added to a tube (V) containing 10 mg. of decalcified sound dentin. A further 2 ml. of the supernatant was transferred to an empty tube (VI). Tubes V and VI were then incubated at 37° C. and assayed for α-NH_2 N liberation at 10-minute intervals for a further 40 minutes.

The results of the experiment are shown in Fig. 6. *I* and *II* show the rates of attack on sound dentin and carious dentin, respectively. *III* shows that a 2:1 mixture of carious and sound dentin is broken down at the rate characteristic of sound dentin alone *(I)*. There is no evidence, therefore, that the presence of an excess of carious dentin affects the ability of collagenase to attack sound dentin at the maximal rate.

The results from V show that following a 40-minute incubation with carious dentin, the collagenase solution will attack sound dentin at the same rate as a collagenase solution that has not been in contact with carious dentin. This too shows that there has been no collagenase inhibitory effect by carious dentin.

Tube VI was set up to determine if there was any marked continuation of α-NH_2 N release when the supernatant was removed from the carious dentin particles. It can be seen that α-NH_2 N liberation almost completely stops when the carious dentin particles have been removed by centrifuging. It may, therefore, be assumed that the rate determined in V is a measure of the action of the enzyme on sound dentin alone, and does not include any continued α-NH_2 N liberation from the earlier incubation with carious dentin. The results from VI also indicate that the action of the B 637 C preparation is upon the carious dentin particles alone, and that there is little further enzyme attack upon the soluble peptide products initially formed.

The experimental results establish that no collagenase inhibitory substance is released into solution in a carious dentin–collagenase incubate.

DISCUSSION

The devolpment of a quantitative method for the estimation of collagenase action on dentin was made necessary by the need for a technic for assessing the relative collagenase susceptibilities of various dentin preparations. The method evolved has proved generally useful, both for studies on sound and carious dentin and on artificially modified dentin.[5] The basis of the method is illustrated in Fig. 7. This diagram is a fragment of a hypothetical collagen polypeptide chain. Each circle represents an amino acid, and the connecting lines indicate the peptide bonds linking the amino acids together to form the polypeptide chain. The arrows indicate supposed sites of collagenase attack. It can be seen that for each peptide bond hydrolyzed

through the catalytic action of the enzyme, an α-amino group (NH_2), and a carboxyl group (COOH) are liberated. It is the estimation of the α-amino group liberation which is used as the index of collagenase attack.

Comparison of the rate of weight loss of dentin particles with the rate of α-NH_2 N release showed that the latter paralleled the rate of solution of dentin particles (see Fig. 3). This establishes the validity of the amino group method as a measure of dentin breakdown. The micro-Kjeldahl nitrogen estimations (see Fig. 4) showed that the fraction assayed for α amino N contained the bulk of the nitrogenous products released by collagenase action, and paper chromatography has shown that this fraction contains peptide fragments resulting from the proteolytic action of collagenase. At least 6 such breakdown products have been detected so far.

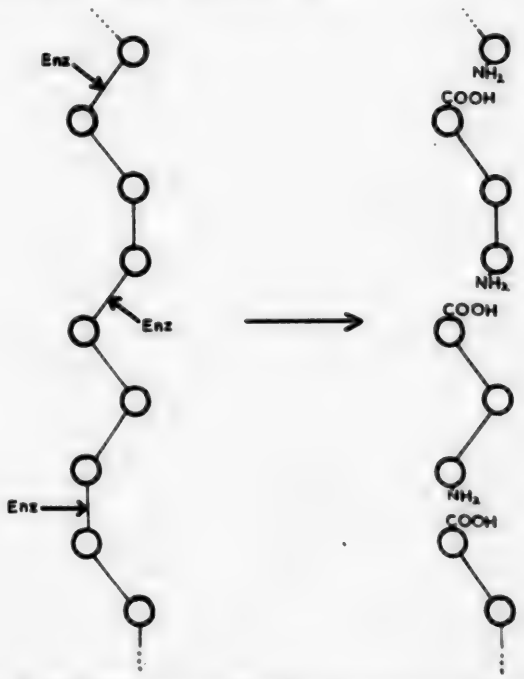


Fig. 7.—Basis of the amino group method for the assay of collagenase action.

On the basis of the antisera experiments it seems fairly certain that the factor present in the B 637 C collagenase preparation responsible for the breakdown of dentin is the "true" collagenase (i.e., κ-toxin). The possibility that some other factor apart from collagenase is involved in the dentin breakdown is made unlikely by the observation that the antiserum RX 5755/36, either alone or in combination with R 1974, had no inhibitory effect upon the activity of the B 637 C preparation in attacking dentin.

Both the agar plate studies and the quantitative investigations (see Fig. 2) have provided evidence that sound dentin decalcified with EDTA is

readily attacked by the collagenase preparations used. In addition, numerous EDTA-decalcified dentin samples have, as a routine procedure, been repeatedly used as a satisfactory standard for comparative studies on the collagenase susceptibilities of various modified dentin preparations.[5]

These observations on the collagenase susceptibility of EDTA-decalcified dentin are at variance with those of Konetzka, Burnett, and Pelczar.[4] The antisera studies provide evidence that the collagenolytic factor in our experiments with B 637 C is κ-toxin (collagenase) as defined antigenically.[13] It is possible that Konetzka and his co-workers may have been dealing with a proteolytic enzyme of different specificity to the collagenases present in the B 637 C preparation or produced by the *Cl. welchii* and *Cl. histolyticum* strains used in this laboratory. It is known, for example, that *Cl. welchii* can produce a proteolytic enzyme (λ enzyme) which attacks denatured or degraded collagen but not native collagen.[14] A similar enzyme is also described for a *Cl. histolyticum* strain.[15] Possibly Konetzka and his associates may have been dealing with a similar type of enzyme in their *Cl. histolyticum* preparations. The strains used by them may well have been of different genetic constitution to those used by Prophet and his associates and ourselves; also they used a somewhat different growth medium. Differing genetic and environmental conditions could have resulted in the production of enzymes of different specificity to those present in our preparations. If Konetzka, Burnett, and Pelczar were using an enzyme similar to the λ-enzyme, this might explain why their preparations attacked only acid-decalcified dentin (presumably degraded to some extent) and not dentin decalcified as physiologic pH. However, it is difficult to explain why their EDTA-decalcified samples did not become susceptible to attack by their enzyme preparations when they subsequently treated them with solutions of low pH.

The application of the method to dentin studies has produced results which substantially confirm the earlier agar plate studies of Evans and Prophet[1, 2] and Prophet and Atkinson.[3] However, the collagenase-resistant property of decalcified carious dentin has been shown to be only partial compared to the totally collagenase-resistant nature of calcified sound dentin (see Fig. 1). There was the possibility that this lowered rate of collagenase attack with carious dentin could be due to the early release of some collagenase inhibiting factor present in the carious dentin. However, experimental evidence (see Fig. 6) has shown that a collagenase solution incubated with carious dentin will subsequently attack decalcified sound dentin at the same rate as a collagenase solution which had not been in contact with carious dentin. This result argues against the possible release of some collagenase-inhibitory substance from carious dentin, and it appears more likely that the collagenase-resistant property of carious dentin is due to some modification of the organic matrix itself.

Experimental evidence identifying specific groups present in the collagen molecule that are related to susceptibility to collagenase attack is reported in a subsequent paper.[5] Investigations into the nature of the changes that have occurred in carious dentin are at present being carried out.

My sincere thanks are due to the Wellcome Research Laboratories for the kind gifts of the collagenase and antisera preparations, and to Mrs. W. Gilchrist for her excellent technical assistance throughout this work.

REFERENCES

1. Evans, D. G., and Prophet, A. S.: Disintegration of Human Dentine by Bacterial Enzymes, *Lancet* **1**: 290, 1950.
2. Evans, D. G., and Prophet, A. S.: Enzymes of Clostridium Welchii Type A and Clostridium Histolyticum That Disintegrate Decalcified Human Tooth Dentine, *J. Gen. Microbiol.* **4**: 36, 1950.
3. Atkinson, H. F., and Prophet, A. S.: The Action of Collagenase on Carious Dentine, *Brit. D. J.* **94**: 278, 1953.
4. Konetzka, W. A., Burnett, G. W., and Pelczar, M. J.: Bacterial Hydrolysis of Decalcified Dentine, *Brit. D. J.* **100**: 156, 1956.
5. Armstrong, W. G.: Modification of the Organic Matrix of Sound Dentin to Collagenase-Resistant Forms, *J. D. Res.* **37**: 1016, 1958.
6. Manly, R. S., and Hodge, C. H.: Density and Refractive Index Studies on Dental Hard Tissues. Methods of Separation and Determination of Purity, *J. D. Res.* **18**: 133, 1939.
7. Yemm, E. W., and Cocking, E. C.: The Determination of Amino-Acids With Ninhydrin, *Analyst* **80**: 209, 1955.
8. Tristram, G. R.: *In* The Proteins, editors, Neurath, H., and Bailey, K., New York, 1953, Academic Press, Vol. 1A, Ch. 3, p. 186.
9. Markham, R.: A Steam Distillation Apparatus Suitable for Micro-Kjeldahl Analysis, *Biochem J.* **36**: 790, 1942.
10. Conway, E. J.: Microdiffusion Analysis, ed. 4, London, 1957, Crosby, Lockwood & Son, Ltd., Ch. 10, p. 99.
11. Consden, R., Gordon, A. H., and Martin, A. J. P.: Qualitative Analysis of Proteins: a Partition Chromatographic Method Using Paper, *Biochem. J.* **38**: 224, 1944.
12. Bidwell, E., and van Heyningen, W. E.: The Biochemistry of Gas Gangrene Toxins. 5. The κ Toxin (Collagenase of Clostridium Welchii), *Biochem. J.* **42**: 140, 1948.
13. Oakley, C. L., Warrack, G. H., and van Heyningen, W. E.: The Collagenase (κ Toxin) of Cl. Welchii Type A, *J. Path. & Bact.* **58**: 229, 1946.
14. Bidwell, E.: Proteolytic Enzymes of Clostridium Welchii, *Biochem. J.* **46**: 589, 1950.
15. Oakley, C. L., and Warrack, G. H.: The Alpha, Beta and Gamma Antigens of Cl. Histolyticum, *J. Gen. Microbiol.* **4**: 365, 1950.

MODIFICATION OF THE ORGANIC MATRIX OF SOUND DENTIN TO COLLAGENASE-RESISTANT FORMS

W. G. ARMSTRONG

Institute of Dental Surgery, University of London, Eastman Dental Hospital, London, W.C. 1, England

INTRODUCTION

IN a previous paper,[1] it was shown that decalcified carious dentin exhibited a partial resistance to attack by collagenase. No evidence was obtained to support the suggestion[2] that decalcification with chelating agents might also involve modification of the organic matrix to a form resistant to collagenase action. Nevertheless, the evidence obtained did support the hypothesis that some alteration of the organic matrix was responsible for the collagenase resistance of carious dentin.

Collagen, which is the main constituent of the organic matrix of dentin, contains about 14 per cent basic amino acids, 18 per cent acidic amino acids and 21 per cent hydroxy amino acids. As a result, the collagen molecule contains a high proportion of side-chains terminating in functional groups of known reactivity (amino, guanidyl, carboxyl, and hydroxyl radicals).

From a knowledge of the mode of action of other proteolytic enzymes, it appeared possible that certain of these side-chain groups would be involved in orientating the collagenase molecule in correct relationship to the specific peptide bonds in the collagen substrate hydrolyzed by the enzyme. If this were so, then the removal of such groups, or their masking by the attachment of specific organic radicals, would be expected to affect the rate of attack by collagenase.

The generally accepted view of the mechanism of enzyme action is that initially an enzyme intimately combines with the substrate to form an intermediary complex. This enzyme-substrate complex is thought to be activated in some way. As a result of this activation the substrate molecule becomes more chemically reactive than it was in the free uncombined state. In more precise terms, the activation energy (energy required for the molecules to react) is lowered for the particular reaction concerned. The reaction proceeds, and the products and enzyme separate.

The outstanding studies of Bergmann and his colleagues established that the presence or absence of certain groups in the substrate molecule, in the neighborhood of susceptible peptide bonds, were major features determining the susceptibility of a substrate to attack by a particular proteolytic enzyme.

Received for publication Dec. 12, 1957; revised by author June 24, 1958.

For example, it was shown, using synthetic peptide substrates, that trypsin would cleave peptide bonds between glycine and lysine units, only if the terminal ε-amino group of the lysine unit was free and unsubstituted. Masking of the ε-amino group by introduction of an organic radical converted a reactive substrate to one resistant to trypsin attack.

It is thought that there are probably several areas or points of attachment involved in the union of substrate and enzyme, and, for an active intermediary complex to be formed, the fit of enzyme to substrate must be exact. In this connection the classical lock and key analogy is useful in visualizing the enzyme-substrate union. Modification of groups known to determine the susceptibility of a particular substrate to enzymic attack may interfere with the correct orientation of enzyme to substrate, or perhaps affect nearby peptide links making them incapable of activation to a more chemically reactive state when combined with the enzyme in an intermediary complex.

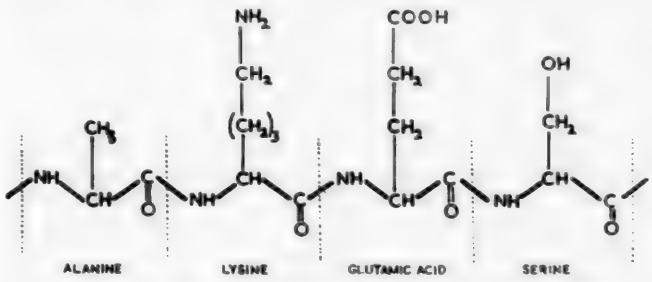
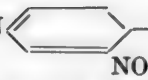


Fig. 1.—Fragment of hypothetical collagen polypeptide chain.

It was thought possible that similar modifications might be responsible for the observed collagenase resistance of carious dentin. It was necessary to establish first whether certain groups in the dentin collagen could determine susceptibility to collagenase attack. This was investigated by reacting dentin with group specific reagents, and then examining the modified dentin preparations for resistance to collagenase. With the information gained it is then possible to investigate whether these groups have been modified in carious dentin.

An example of the reaction of dentin with a group specific reagent is illustrated in the following diagrams. Fig. 1 shows a fragment of a hypothetical collagen molecule. The backbone of the polypeptide chain is made up of the peptide bonds ($-\overset{\overset{O}{\|}}{C}-\overset{\overset{H}{|}}{N}-$) linking the individual amino acid units together. The methyl (CH_3) side-chain of the alanine unit is very unreactive, but the amino (NH_2) group of the lysine units, the carboxyl (COOH) group of the glutamic acid, and the hydroxy (OH) group of the serine unit are potentially reactive.

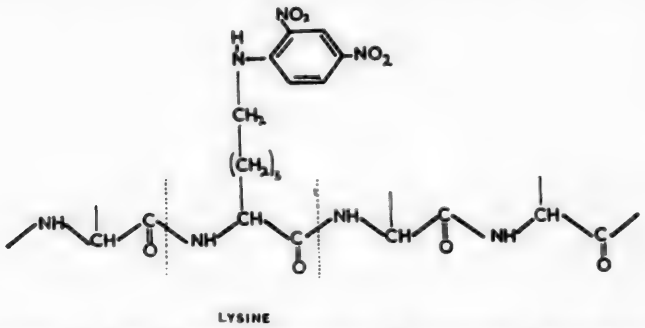
The reagent fluorodinitrobenzene specifically reacts with the terminal amino groups of lysine (and hydroxylysine) units as indicated in Fig. 2.

The introduction of the bulky dinitrophenyl radical (O_2N  NO_2) may interfere with some collagenase anchoring or orientating role of the amino group. Alternatively, its positioning may "protect" nearby peptide links from enzyme action.

MATERIALS AND METHODS

Preparation of Dentin.—

Sound dentin particles were prepared as described previously.[1] They were decalcified with 20 per cent ethylenediamine-tetraacetic acid (EDTA) at pH 7.4, washed 5 times with water, 3 times with acetone, and air dried. Each modified dentin was prepared from this stock except where otherwise stated.



LYSINE

Fig. 2.—Illustration of action of fluorodinitrobenzene in specifically blocking the terminal (ε) amino group of the lysine unit in collagen polypeptide chain.

Modified Dentin Preparations.—

Deaminated dentin (DD): The deamination was effected using an adaptation of the diazotization procedure of Thomas and Foster[3] as reported by Gustavson.[4] To 10 ml. of water, 0.2 Gm. of decalcified dentin was added. After 30 minutes, 5 ml. of 20 per cent $NaNO_2$ was added and the suspension was stirred for 10 minutes and then allowed to stand overnight. With constant stirring, 0.7 ml. of glacial acetic acid was added dropwise. The suspension was stirred overnight, and then centrifuged. The dentin product, which was pale yellow, was washed 4 times with 10 per cent NaCl, 5 times with water, and finally 3 times with acetone. It was air dried and stored in the dark. A control DD was similarly prepared, substituting 20 per cent NaCl for the 20 per cent $NaNO_2$ solution. Quantitative analysis of DD hydrolysates by ion-exchange chromatography showed that 82.7 per cent lysine and 64 per cent hydroxylysine had been deaminated.

N-acetylated dentin (NAD): One-tenth gram of decalcified dentin was soaked overnight in half saturated sodium acetate solution at 5° C. One-tenth of a milliliter of acetic anhydride was added with stirring and the pH maintained near 8.0 by addition of alkali. The suspension was stirred for 1 hour,

then centrifuged, and repeatedly washed with water. It was finally washed 3 times with acetone and air dried. A control NAD was similarly treated, omitting the addition of acetic anhydride. The acetylation procedure employed was an adaptation of that reported by Gustavson.[4] Analysis of NAD for total N-acetyl by the method of Green, Ang, and Lam[6] showed that 62.5 per cent of the ϵ-NH_2 groups had been acetylated. No O-acetyl was detected.

Dinitrophenylated dentin (DNP-D): Two-tenths of a gram of decalcified dentin and 0.2 Gm. $NaHCO_3$ were added to 2.5 ml. water; 5 ml. of a 5 per cent ethanolic solution of 1-fluoro-2, 4 dinitrobenzene (FDNB) were then added. The suspension was shaken at room temperature for 2 hours. It was then acidified with a few drops of concentrated HCl, shaken with 3 successive 25 ml. portions of ether and finally shaken with 25 ml. of ethanol and a further 25 ml. of ether. The bright yellow product was air dried. The control was similarly treated but excluded the addition of FDNB. The dinitrophenylation procedure employed was an adaptation of the original Sanger technic.[5] Hydrolysates of DNP-D were analyzed for total DNP-lysine and DNP-hydroxylysine,[13] and the results showed that 71.4 per cent of the lysine and hydroxylysine had been converted to DNP derivatives. Quantitative analysis of DNP-D hydrolysates by ion-exchange chromatography showed that 14.6 per cent lysine and 23.6 per cent hydroxylysine had not reacted with FDNB.

Methylated dentin (MD): Two-tenths gram of decalcified dentin was shaken intermittently for 7 days in 20 ml. of methanol made 0.1 N with respect to HCl. It was then repeatedly washed with water, thrice dehydrated with acetone, and air dried. The product was light brown. A control was prepared by standing a dentin sample in methanol for 7 days. The method is that recommended by Gustavson.[4] A comparison of the acid-binding capacities of MD and untreated dentin showed that 89 per cent of the free carboxyl group content had been esterified in MD.

N- and O-acetylated dentin (NOAD): One-tenth gram of decalcified dentin was added to 0.6 ml. of a 1:1 mixture of glacial acetic acid and acetic anhydride. It was stored for 8 days at room temperature. The product was thoroughly washed with water, thrice dehydrated with acetone, and finally air dried. A control was prepared by suspending dentin in glacial acetic acid for 8 days. The method is that developed by Green, Ang, and Lam[6] as reported by Gustavson.[4] Using the analytical procedures of Green and coworkers[6] is was found that 32.6 per cent of the ϵ-NH_2 and 68.8 per cent of the total hydroxy group content had been acetylated in NOAD.

Sulfated dentin (SD): One-tenth gram of decalcified dentin was placed in a boiling tube in a Dewar flask containing an acetone solid CO_2 mixture at –40° C. Concentrated sulfuric acid was cooled to just above its crystallization point, and 2 ml. added dropwise to the dentin. The tube was then transferred to an ice-bath and allowed to rise slowly to room temperature (2 hr.). The suspension was then poured onto finely crushed ice in a beaker. The tube, which still contained a quantity of the suspension adhering to the wall, was packed

with finely crushed ice. After 5 minutes the contents of the tube was washed into the beaker with ice-cold water. Alkali was added to neutralize the acid, and the dentin product thrice washed with water, then thrice dehydrated with acetone. It was air dried. The amount of sulfate present in hydrolysates of SD and untreated dentin was determined by the method of Dodgson and Spencer.[14] The increase in sulfate content of SD over untreated dentin corresponded to a 97.3 per cent esterification of the total hydroxy groups present.

Deguanidated dentin (DGD): Two-tenths gram of decalcified dentin was suspended in 10 ml. distilled water and cooled in an ice-bath. Twenty milliliters of a saturated bromine solution was mixed with 10 ml. of 10 N NaOH; a 1/5 dilution of this hypobromite solution was prepared with distilled water and cooled to 0° C. Ten milliliters of this solution was added slowly, over 2 hours, to the ice-cold dentin suspension, which was continually stirred throughout this period. The product was twice washed with ice-cold water and once with 0.5 N acetic acid. It was then soaked overnight with 0.5 N acetic acid at 2° C. It was next washed 4 times with water, 3 times with acetone, and then air dried. The control was prepared by subjecting a dentin sample to the same additions of the alkali and acetic acid solutions used for the DGD preparation. Ion-exchange chromatographic analysis of a DGD hydrolysate showed that all of the arginine had been attacked.

Guanidated dentin (GD): One-tenth gram of decalcified dentin was added to 20 ml. of an ice-cold 0.1 M solution of S-methylisothiourea sulfate, adjusted to pH 10.5 with NaOH. It was stored in the refrigerator at 0° C. for 3 days, with occasional shaking. The suspension was then diluted to 200 ml. and the GD collected by centrifuging. It was repeatedly washed with water, then thrice dehydrated with acetone, and finally air dried. The GD control was prepared by suspending dentin in 0.001 N NaOH at 0° C. for 3 days. The procedure used was an adaptation of that reported by Hughes, Saroff, and Carney.[7] Analysis of GD hydrolysates by a modified Sakaguchi procedure[15, 16] showed an increase in guanidyl content corresponding to reaction of 98 per cent of the total lysine and hydroxylysine content. A subsequent analysis, by ion-exchange chromatography, of the hydrolysate showed that 96 per cent of the total lysine had reacted to form the corresponding homo-arginine analogue.

Formol-saline treated dentin: Three preparations of formol-saline treated dentin were made:

1. FD1. 0.1 Gm. decalcified dentin was added to 10 ml. 5 per cent formol-saline, and stood at room temperature, with intermittent shaking, for 7 days. The product was then thoroughly washed with water, thrice dehydrated with acetone, and air dried.
2. FD2. 0.5 Gm. undecalcified dentin was similarly treated with formol-saline. After a thorough washing the particles were decalcified with EDTA and then washed, acetone dehydrated, and air dried.

3. FD3. Sound teeth which had been stored in 5 per cent formol-saline for 4 weeks were thoroughly washed. Dentin particles were then prepared and decalcified as described for freshly extracted teeth.

"Tea-tanned" dentin (TTD): A strong tea infusion was prepared by boiling 5 parts M/15 Sorensen buffer, pH 7.4, with one part of tea (w/w) for 10 minutes. After cooling, the supernatant was collected and 10 ml. added to 0.1 Gm. of EDTA-decalcified dentin. The same volume of tea infusion was

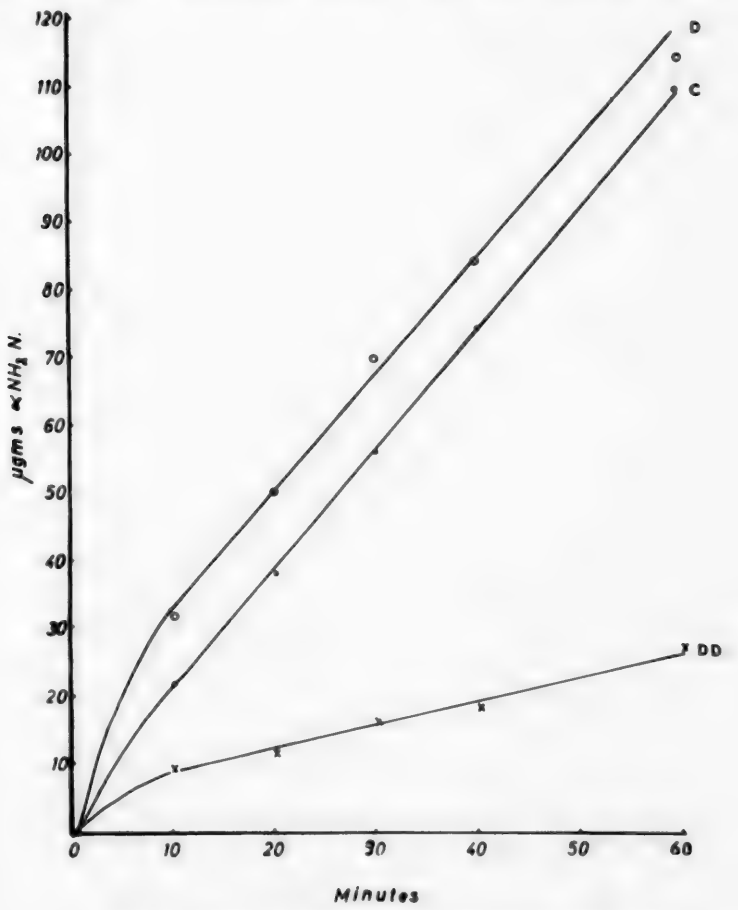


Fig. 3.—Action of collagenase on deaminated dentin (*DD*).

added to 0.5 Gm. undecalcified dentin. The suspensions were kept at room temperature for 4 days with intermittent shaking. Thymol crystals were added to inhibit microbial growth. The "tea-tanned" dentin samples were then repeatedly washed with water until the supernatants were colorless. They were then washed with acetone until the supernatants were again

colorless. The calcified dentin was decalcified with 20 per cent EDTA at pH 7.4, thoroughly washed and acetone dehydrated. Both products were air dried.

Collagenase Solutions.—

All dentin preparations were tested for susceptibility to collagenase attack with 30 mg. per cent (approx.) solution (pH 7.4) of a partially purified collagenase preparation (B 637 C) supplied by the Wellcome Research Laboratories.

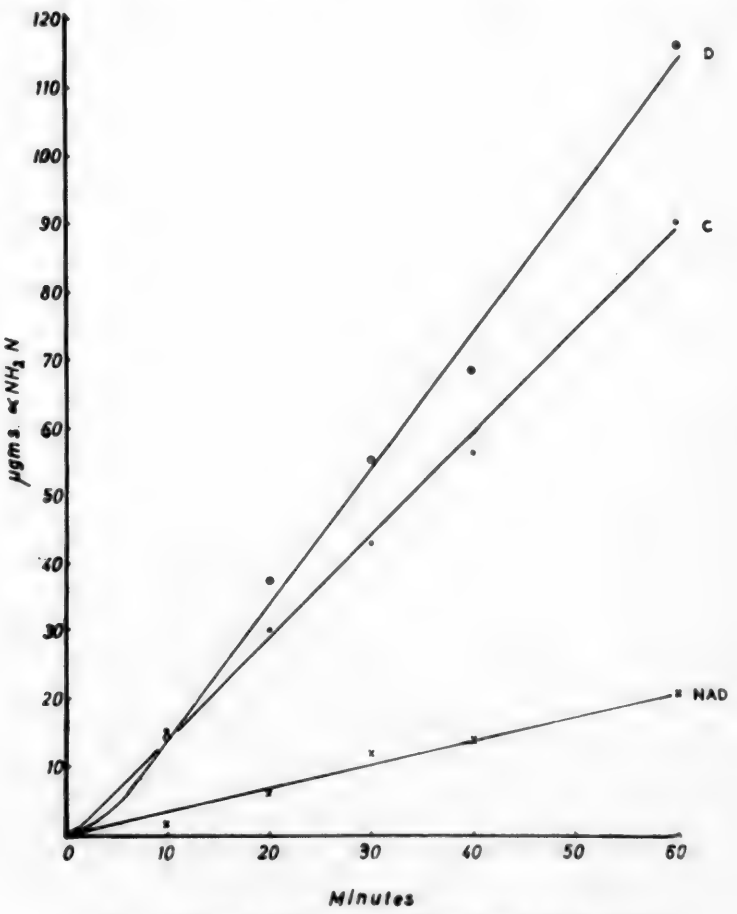


Fig. 4.—Action of collagenase on N-acetylated dentin (*NAD*).

Estimation of collagenase action: Collagenase action was measured by determining the rate of α-NH_2 N liberation, using the method previously described.[1]

EXPERIMENTAL AND RESULTS

The susceptibility to attack by collagenase of each modified dentin was compared directly with that of an untreated dentin sample (D) examined at

the same time. It was possible that any of the stages in the preparation of a particular modified dentin might have altered the collagenase susceptibility of the matrix. To investigate this possibility, control dentin samples (C) were prepared, where applicable, by eliminating the addition of an essential reagent, but otherwise subjecting the dentin to the same procedures involved in the preparation of the particular modified dentin concerned. The rate of attack by collagenase on each control dentin sample was determined at the same time as the estimations on the untreated and modified dentin samples.

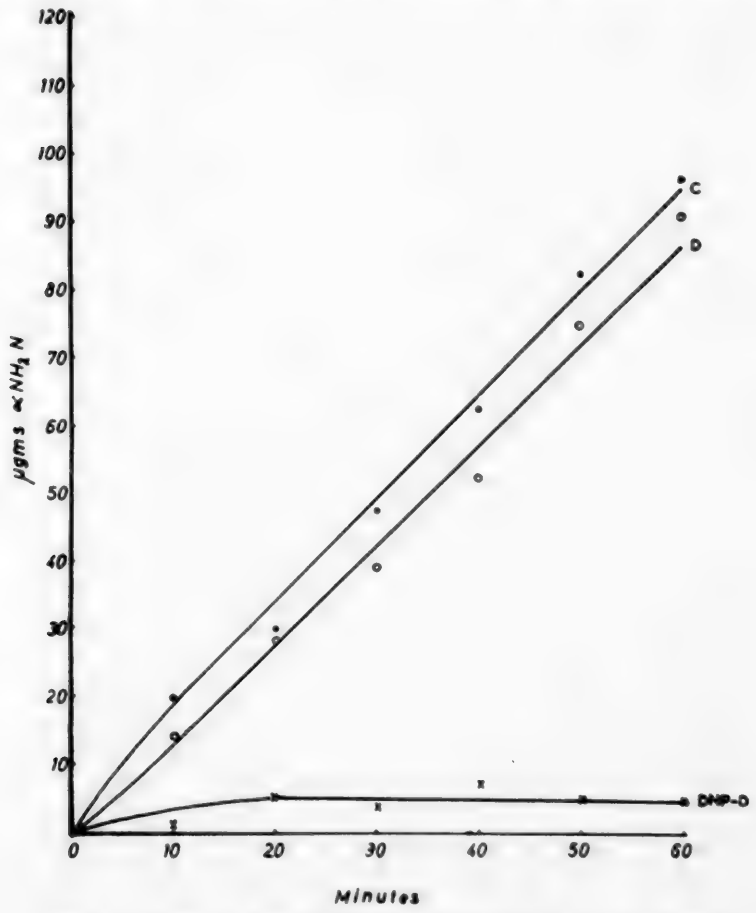


Fig. 5.—Action of collagenase on dinitrophenylated dentin (*DNP-D*).

In addition, investigations were made to determine whether there was any α-NH_2 N release when each modified dentin was incubated in the presence of buffer alone. No evidence for such breakdown was obtained; similarly, no evidence of α-NH_2 N release was found in enzyme-buffer incubates. These negative results have not been included in Figs. 3 to 12.

I. *Effect of Modification of ϵ-NH_2 Groups Present in Dentin Collagen.*—

1. *Removal of ϵ-NH_2 groups by diazotization:* The deaminated dentin sample (DD) was examined for its susceptibility to collagenase attack. The results, which are given in Fig. 3, show that DD was broken down at approximately one fifth of the rate of the untreated decalcified dentin and the DD control.

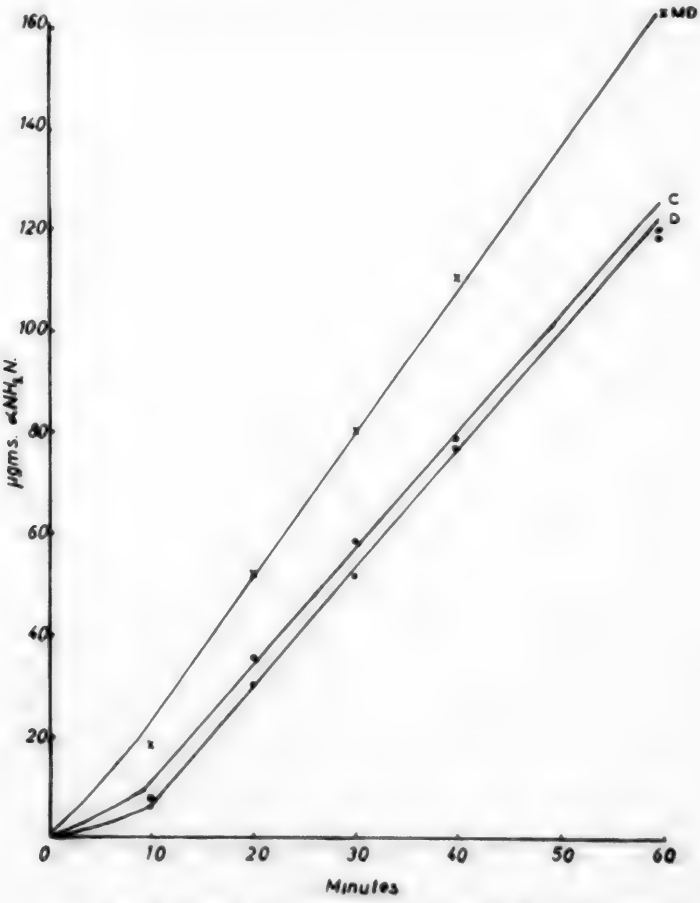


Fig. 6.—Action of collagenase on methylated dentin (*MD*).

2. *Acetylation of ϵ-NH_2 groups:* The N-acetylated dentin (NAD) was similarly tested against collagenase. The results (Fig. 4) show that NAD, like the DD sample, was broken down at about one fifth of the rate of the untreated substrate. The NAD control also exhibited a small degree of resistance to collagenase.

3. *Dinitrophenylation of ϵ-NH_2 groups:* The dinitrophenylated dentin (DNP-D) showed initially a very small amount of α-NH_2 N liberation in the

presence of collagenase, but after 30 minutes this stopped completely (Fig. 5). In a separate experiment a 12-hour acid hydrolysis of the DNP-D, followed by examination of the products by paper chromatography,[8] showed that only two DNP-amino acids were present in the acid hydrolysate. The major component was identified as a DNP-lysine and the smaller one was assumed to be DNP-hydroxylysine.

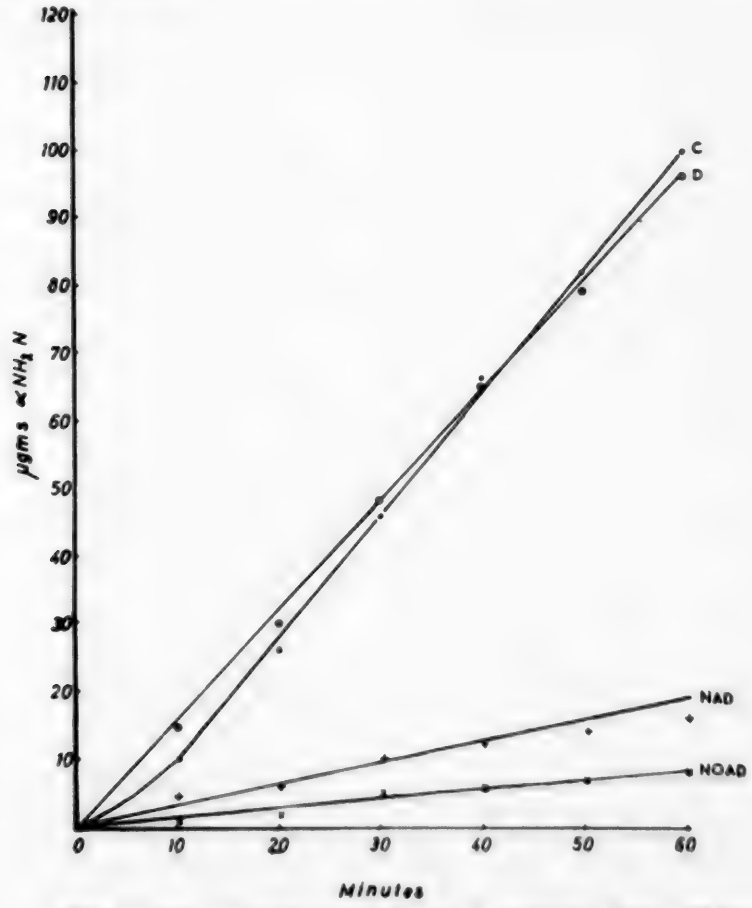


Fig. 7.—Action of collagenase on N- and O- acetylated dentin (*NOAD*).

II. *Effect of Modification of Carboxyl Groups Present in Dentin Collagen.*—

1. *Methylation of terminal carboxyl groups of glutamic acid and aspartic acid residues:* The measurement of the rate of attack by collagenase of methylated dentin (MD) is shown in Fig. 6. The MD is attacked at a somewhat higher rate than the untreated dentin.

III. *Effect of Modification of Free Hydroxy Groups Present in Dentin Collagen.*—

1. *Acetylation of ϵ-NH_2 and OH groups:* Acetylation of dry collagen with the nonaqueous acetylating medium, 1:1 acetic anhydride/glacial acetic acid, affects both the free amino (NH_2) groups and the hydroxy (OH) groups. The results in Fig. 7 show that N- and O-acetylated dentin (NOAD) was attacked by collagenase at a lower rate than an N-acetylated dentin sample examined concurrently.

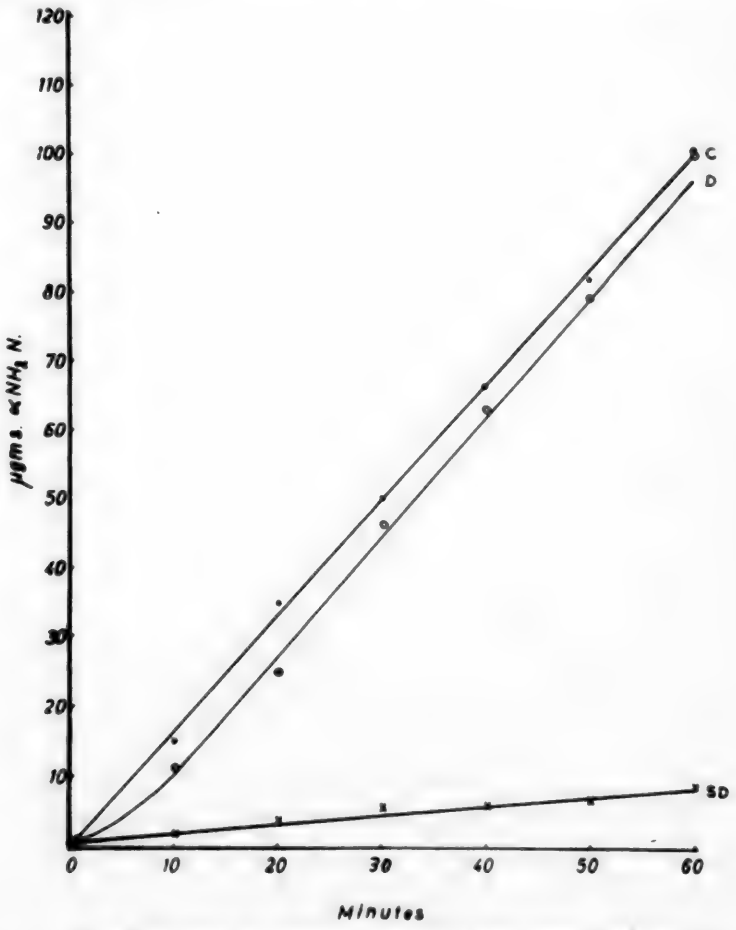


Fig. 8.—Action of collagenase on sulfuric acid-treated dentin (*SD*).

2. *Esterification of hydroxy groups with concentrated sulfuric acid:* The sulfuric acid treated dentin (SD) showed a marked resistance to attack by collagenase (Fig. 8).

IV. *Effect of Destruction and Introduction of Guanidyl Groups in Dentin Collagen.*—

1. *Reaction with sodium hypobromite solution:* Hypobromite in alkaline solution attacks and destroys the terminal guanidyl groups of the arginine

residues present in the collagen molecule. Fig. 9 shows the result of such treatment upon the collagenase susceptibility of the organic matrix of dentin. The rate of attack of collagenase on deguanidated dentin (DGD) was found to be low.

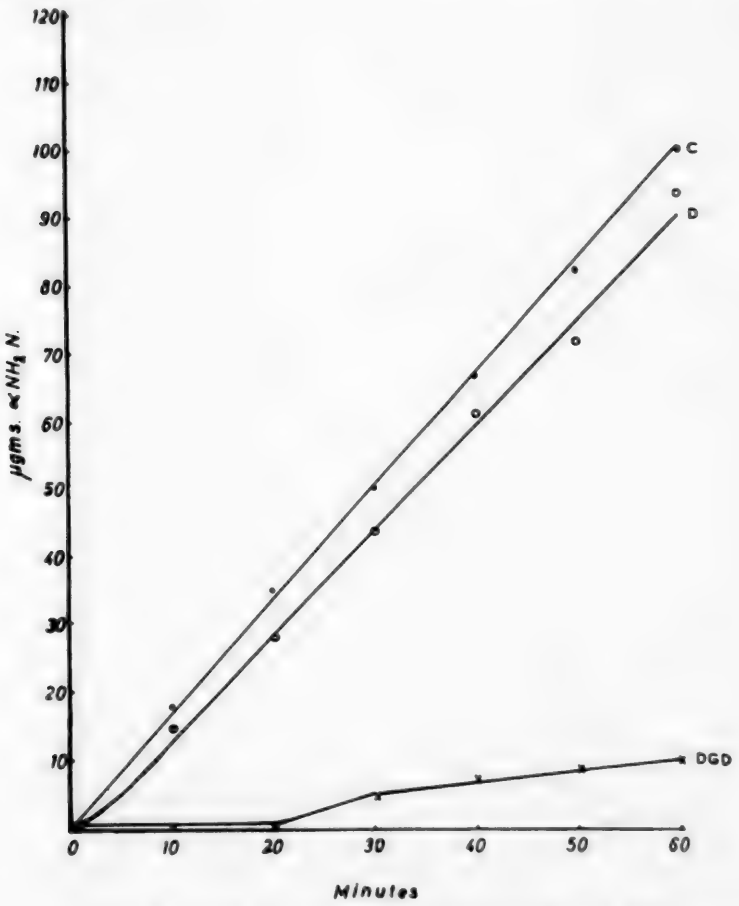


Fig. 9.—Action of collagenase on deguanidated dentin (*DGD*).

2. *Reaction with S-methylisothiourea:* This reagent reacts with the ϵ-NH_2 groups present in collagen converting them to guanidyl groups. The effect of this alteration upon the susceptibility to collagenase attack of the organic dentin matrix is shown in Fig. 10. Initially, the modified dentin was attacked at the same rate as untreated dentin, but after 30 minutes the rate fell sharply to about one third of this value.

V. *Effect of Tanning Agents on Dentin Collagen.*—

1. *Formol-saline:* Treatment of decalcified or undecalcified dentin particles with 5 per cent formol-saline converted the organic matrix of dentin to a form

completely resistant to collagenase attack (*FD1* and *FD2* in Fig. 11). In addition, dentin particles prepared from teeth which had been stored in formol-saline were shown to be almost completely resistant to the enzyme (*FD3* in Fig. 11).

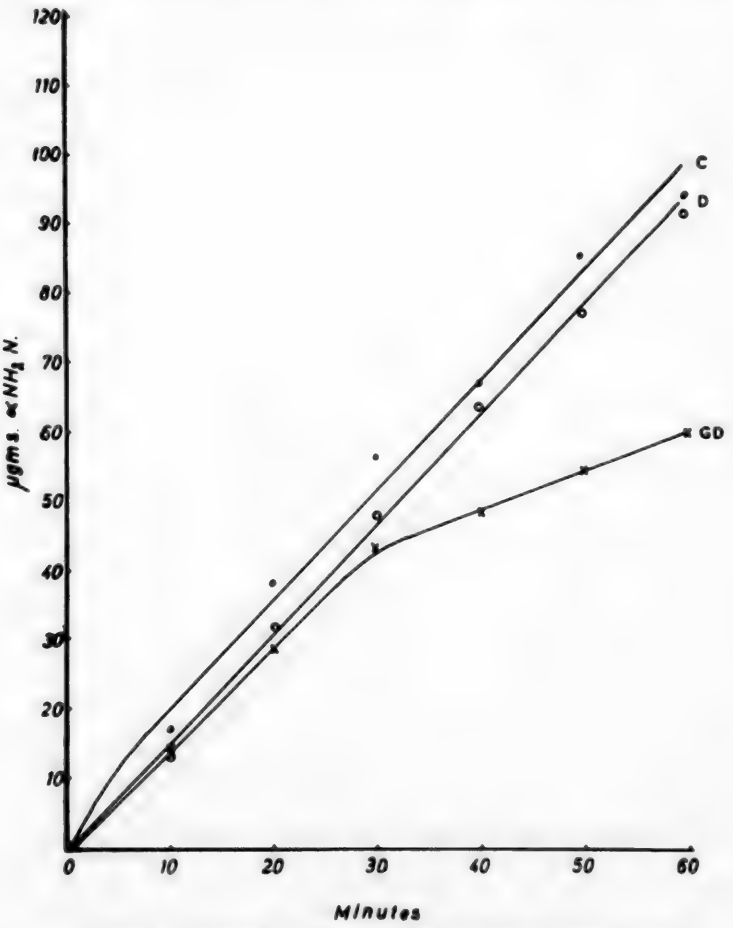


Fig. 10.—Action of collagenase on guanidated dentin (*GD*).

2. *Tea infusion:* Treatment of undecalcified dentin particles with a tea infusion converted the organic matrix to a form that was only slightly attacked by collagenase (Fig. 12). Similar treatment of decalcified dentin particles made them completely resistant to collagenase.

DISCUSSION

The experimental results given above show that modification of the organic matrix of dentin by group specific reagents can alter its susceptibility to collagenase attack. These reagents have been widely used in other fields

of collagen chemistry and are known to be relatively specific in their action. The possibility cannot be excluded that unanticipated side reactions of a physical or chemical nature may have occurred to a limited extent in some preparations, and such unknown modifications might contribute in part to any

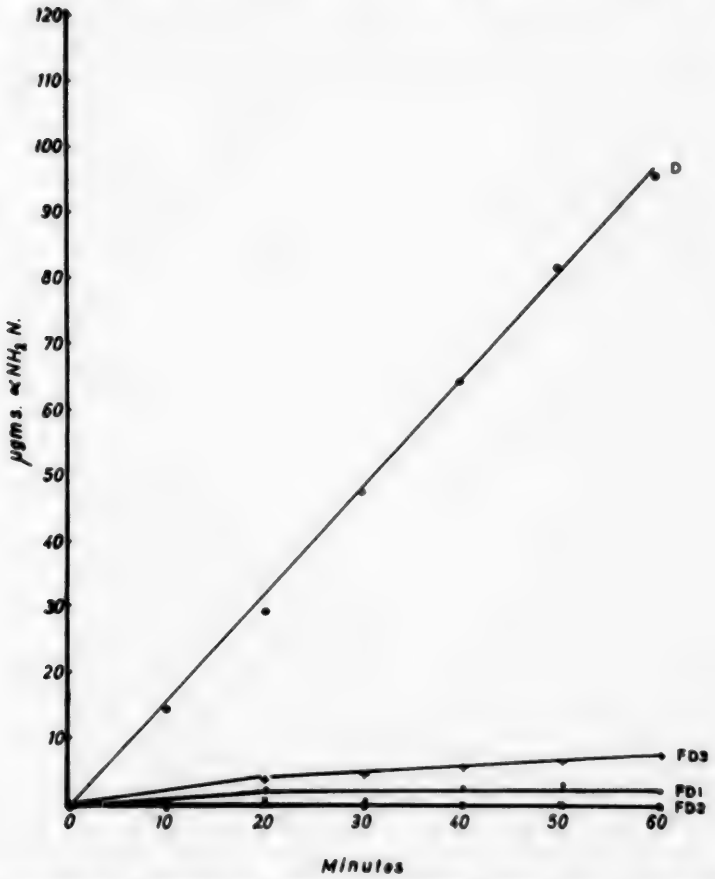


Fig. 11.—Action of collagenase on formol-saline treated dentin (*FD1, FD2, FD3,* see text).

observed change in the collagenase susceptibility of a particular dentin preparation. However, the analytical data show that in each modified dentin preparation a major modification of the particular group(s) aimed at had occurred, and it is these known changes which, it is considered, are primarily responsible for any observed change in the properties of the matrix as a whole.

Removal of the ε-amino groups of the lysyl and hydroxylysyl residues on dentin collagen considerably reduced its rate of breakdown by collagenase (Fig. 3). A similar degree of collagenase resistance was found when the ε-amino groups were acetylated (Fig. 4), and complete resistance was shown with dinitrophenylated dentin (Fig. 5). The collagenase-resistant property

of acetylated dentin and dinitrophenylated dentin could be explained either as being due to the masking of collagenase anchoring or orientating sites, or as the result of a steric hindrance effect protecting susceptible peptide bonds. However, the fact that deaminated dentin is collagenase resistant indicates

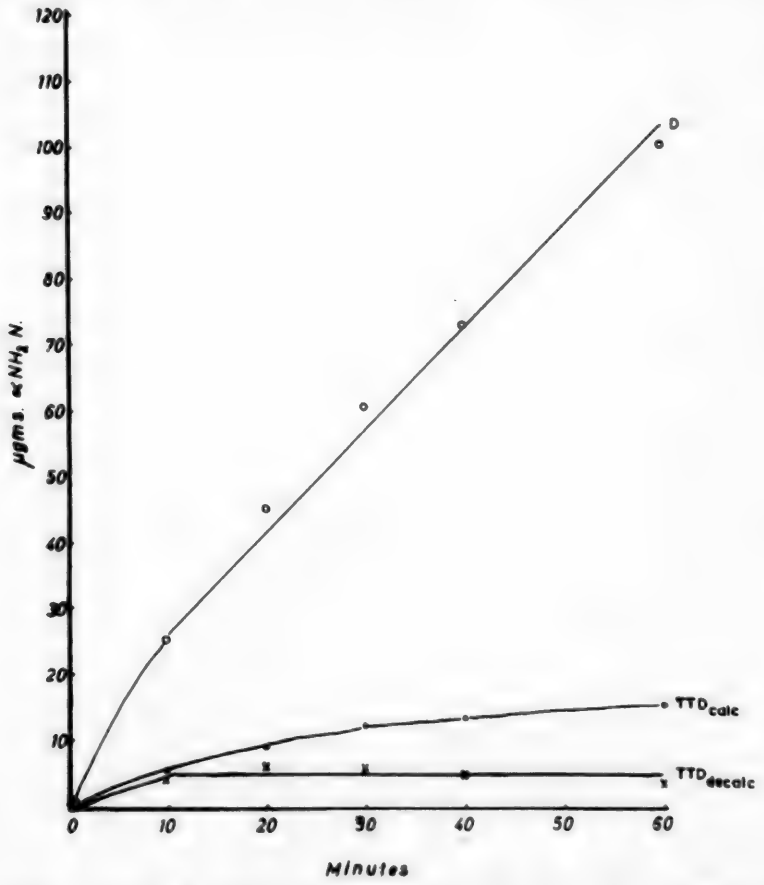


Fig. 12.—Action of collagenase on tea-tanned dentin (*TTD calc.* and *TTD decalc.*).

that it is likely that the role of the ϵ-amino groups is in anchoring or orientating the enzyme in relation to collagenase-susceptible peptide bonds, or that their presence is in some way related to a subsequent activation of these bonds.

Masking of the γ-carboxyl groups of the aspartyl and glutamyl residues by esterification with methanol does not increase the resistance of dentin collagen to collagenase attack (Fig. 6). Indeed, as the results show, the methylated dentin is attacked at a higher rate than untreated dentin. This probably reflects other changes that occurred in the dentin collagen

during the methylation procedure; the 0.1 N HCl methanolic solution may have caused breaking of ester cross-links and hydrogen bonds, which are of importance as interchain and intermolecular linkages in collagen.

The investigation into the possible role of hydroxy groups in collagenase susceptibility indicated that they were probably involved. It was thought that if hydroxy groups were of importance in this connection, then dentin collagen with both the amino groups and the hydroxy groups acetylated would be more resistant to collagenase than a modified dentin with only the amino groups acetylated. The results (Fig. 7) showed that the N- and O-acetylated dentin (NOAD) was, in fact, more resistant to collagenase than N-acetylated dentin (NAD). The rate of collagenase attack on NAD was about 20 per cent of that on untreated dentin; with NOAD the rate of attack was about 8 per cent of the normal. Since NAD was itself very resistant to collagenase, the effect of any additional modification tending to further increase this property would be detected only as a small fraction of the total resistance, and it was not possible to assess the relative importance of the hydroxy groups in determining collagenase susceptibility. It was therefore necessary to study a modified dentin in which the hydroxy groups alone had been altered.

It has been reported[9] that at low temperatures concentrated sulfuric acid attacks selectively the hydroxy groups in proteins to form acid sulfate esters, and, when the organic matrix of dentin was treated with concentrated sulfuric acid, it was converted to a product (*SD*—Fig. 8) which was highly resistant to collagenase. If concentrated sulfuric acid acts only by esterifying the hydroxy groups present in the organic matrix, then this result may be interpreted as evidence that hydroxy groups are of considerable importance in determining susceptibilty to collagenase attack. However, it appears conceivable that sulfuric acid might modify the physical and chemical properties of collagen in various ways. In addition, there is Gustavson's failure to utilize the reaction successfully in certain of his studies on collagen.[4] Hence the results from sulfuric acid treated dentin should perhaps be interpreted with some caution. Nevertheless, the results from the studies on NOAD and SD do indicate the likelihood that hydroxy groups play some role in determining the collagenase susceptibility of the organic matrix of dentin.

The terminal guanidyl groups of the arginine residues also appear to be related to collagenase susceptibility. Destruction of the guanidyl groups produced a modified dentin highly resistant to the enzyme (Fig. 9).

The effect of the guanidation of the ϵ-amino groups in dentin collagen using S-methylisothiourea is of considerable interest. First, the reaction may be regarded as an ϵ-amino group masking process but, unlike the other reactions used, this modification of the ϵ-amino groups does not markedly reduce the basic property of the ends of the amino acid side chains involved (the basic ϵ-amino groups are changed to basic guanidyl groups). Second, the reaction increases the total free guanidyl content of the organic dentin matrix (the lysine and hydroxylysine residues are converted, in situ, to their corresponding homo-arginine analogues). The presence of free guanidyl groups has

been shown (Fig. 9) to influence the collagenase susceptibility of the dentin matrix, and it was possible that introduction of additional guanidyl groups could increase the rate of collagenase attack.

It might therefore be anticipated that reaction of decalcified dentin with S-methylisothiourea would increase resistance to collagenase by virtue of the ϵ-amino group masking effect. There is also the possibility that this effect would be reversed to some extent because of the retention of the basic nature of the end group, and because of the increase in total free guanidyl groups throughout the matrix. The results in Fig. 10 show the effect of introducing further guanidyl groups in dentin collagen. The initial rate of breakdown (Fig. 10), which is equal to that of untreated dentin, probably means that although guanidation of the ϵ-NH_2 groups was quantitative, there are still present collagenase-susceptible sites accessible to enzyme attack. If certain regions of the guanidated dentin collagen still possessed such collagenase-susceptible sites, it is reasonable to assume that the collagenase would attack these regions unhindered and, therefore, at the normal rate. After all these sites had been attacked, any subsequent lowered rate of attack would be a measure of an altered collagenase susceptibility in the remaining part of the molecule. As shown in Fig. 10, the rate of α-NH_2 liberation from GD/collagenase incubates fell sharply, after 30 minutes, to a value that was about one third that of the untreated dentin, but about 70 per cent greater than deaminated or N-acetylated dentin. This rate (Fig. 10) is considered to represent the true measure of the effect of guanidation of dentin upon its susceptibility to collagenase attack. For the reasons given above, it appears conceivable that its value could be interpreted as a measure of the balance between factors tending to reduce the rate of enzyme attack on dentin collagen (masking of ϵ-amino groups) and factors related to susceptibility to enzyme attack (basic end groups of modified lysine side-chains; increase in total free guanidyl group content).

So far, the reagents used for the production of the various modifications of dentin investigated have been relatively specific in action. With the formol-saline treated teeth and the tea-tanned dentin, the nature of the changes induced in the dentin by them is less certain. Formaldehyde is known to attack the free amino groups present in proteins but, in addition, it may form methylene cross links between these amino groups and amide, guanidyl, or imidazole groups, so increasing the stability of the intermolecular bonding between collagen chains.

Similarly, vegetable tannins, such as those found in tea infusions, are thought to form intermolecular cross linkages in collagen. It is known that the basic groups are involved in the reactions of collagen with vegetable tannins, although opinions differ on the relative importance of this in the overall tanning effect.[10-12]

The experimental results from the formaldehyde and tea infusion studies (Figs. 11, 12) establish that treatment of the organic matrix of dentin with either of these reagents modifies it to a collagenase-resistant form. In view

of the established role of free ε-amino groups and guanidyl groupings in collagenase susceptibility, the involvement of these groups in formaldehyde and vegetable tanning reactions is of considerable interest. An important fact to emerge from these tanning studies is that the organic matrix of dentin can be modified to a collagenase-resistant form even in extracted, fully calcified teeth. The completely collagenase-resistant property of fully calcified dentin could lead to the view that the organic matrix was relatively inaccessible in the calcified state. It may be that this is true for the peptide bonds broken by collagenase, but it now appears certain that sites which determine the rate of attack by collagenase are available for reaction in the fully calcified matrix. It follows that there is the possibility that the observed collagenase-resistant property of carious dentin may have been acquired prior to any decalcification mechanism. Whether this does occur during the carious process and if it is of importance are, at present, matters for conjecture. Its significance would depend upon the extent to which the structure and metabolism of the organic matrix is involved in the development of caries of the dentin. An investigation is currently being carried out to determine whether the collagenase-resistant nature of carious dentin is due to modifications similar in principle to those occurring in the chemically altered dentin samples which show collagenase resistance.

SUMMARY

The organic matrix of sound dentin is readily disintegrated by collagenase, and it has been shown that the presence of free ε-amino and guanidyl groups in the matrix determines susceptibility to attack by the enzyme. It is likely that hydroxy groups are similarly involved.

Modification of the decalcified organic matrix of sound dentin with specific reagents can alter the matrix to collagenase-resistant forms. It is also possible to similarly modify the matrix without preliminary decalcification.

The significance of the results is discussed in relation to the observed collagenase resistance found in carious dentin.

I am indebted to the Wellcome Research Laboratories for their kind gift of B 637 C collagenase preparations. My sincere thanks are due to Mrs. W. Gilchrist for her invaluable technical assistance throughout this work.

REFERENCES

1. Armstrong, W. G.: Further Studies on the Action of Collagenase on Human Sound and Carious Human Dentin, *J. D. Res.* **37**: 1001, 1958.
2. Konetzka, W. A., Burnett, G. W., and Pelczar, M. J.: Bacterial Hydrolysis of Decalcified Dentine, *Brit. D. J.* **100**: 156, 1956.
3. Thomas, A. W., and Foster, S. B.: The Behavior of Deaminized Collagen; Further Evidence in Favor of the Chemical Nature of Tanning, *J. Am. Chem. Soc.* **48**: 489, 1926.
4. Gustavson, K. H.: The Chemistry and Reactivity of Collagen, New York, 1956, Academic Press, ch. 10.
5. Sanger, F.: The Free Amino Groups of Insulin, *Biochem. J.* **39**: 507, 1945.
6. Green, R. W., Ang, K. P., and Lam, L. C.: Acetylation of Collagen, *Biochem. J.* **54**: 181, 1953.
7. Hughes, W. L., Saroff, H. A., and Carney, A. L.: Preparation and Properties of Serum and Plasma Proteins. XXII. A Crystallizable Guanidated Derivative of Human Serum Albumin, *J. Am. Chem. Soc.* **71**: 2476, 1949.

8. Biserte, G., and Osteux, R.: La chromatographie de partage sur papier des dinitrophenyl-amino acides, *Bull. Soc. chim. biol.* **33**: 50, 1951.
9. Reitz, H. C., Ferrel, R. E., Fraenkel-Conrat, H., and Olcott, H. S.: Action of Sulfating Agents on Proteins and Model Substances. I. Concentrated Sulfuric Acid, *J. Am. Chem. Soc.* **68**: 1024, 1946.
10. Bowes, J. H., and Kenten, R. H.: The Effect of Deamination and Esterification on the Reactivity of Collagen, *Biochem. J.* **44**: 142, 1949.
11. Lollar, R. M., and Kremen, S. S.: Studies of Vegetable Tannage. VI. The Comparative Fixation of Ligno-sulfates and True Tannins, *J. Am. Leather Chem. Assoc.* **43**: 542, 1948.
12. Lipsitz, P., Kremen, S. S., and Lollar, R. M.: Studies of Vegetable Tannage. VIII. The Comparative Fixation of Commercial and Purified Vegetable Tanning Materials, *J. Am. Leather Chem. Assoc.* **44**: 371, 1949.
13. Bowes, J. H., and Moss, J. A.: The Reaction of Fluorodinitrobenzene With the α- and ϵ-Amino Groups of Collagen, *Biochem. J.* **55**: 735, 1953.
14. Dodgson, K. S., and Spencer, B.: Studies on Sulphatases, 5, *Biochem. J.* **55**: 436, 1953.
15. MacPherson, H. T.: The Basic Amino Acid Content of Proteins, *Biochem. J.* **40**: 470, 1946.
16. Janus, J. W.: Determination of Arginine, *Nature* **177**: 529, 1956.

CONTINUOUS CEMENTUM APPOSITION

H. A. ZANDER AND BEAT HÜRZELER

Department of Periodontology, Eastman Dental Dispensary, Rochester, N. Y.

INTRODUCTION AND REVIEW OF LITERATURE

THE earliest recognition of cementum probably dates back to 1667 (Malpighi).[1] As early as 1878, Magitot[2] stated that cementum appears to increase in thickness with age. He talked about a "continuous increase in volume." G. V. Black[3] compared teeth from people aged 30 years with those of age 50 and a third group aged 70. In view of his observations, he states that cementum grows continuously. Since his time many authors[4-8] have stated the same thing. Gottlieb[9, 10] stressed continuous deposition of cementum as an indication of cementum vitality and created the hypothesis that periodontal disease may be primarily a disease of the cementum (cementopathia).[11] Several authors have related cementum formation to function.[12-20]

Wiebusch[21] stated recently, "For some years the writings on cementum have emphasized that deposition of this hard tissue takes place continually throughout life. More current theories suggest that continuous deposition of cementum on the root surface may not be a normal, physiologic function and should perhaps be considered an atypical observation. Which of these two theories is valid, of course, is open to debate. The solution of this problem may be offered after the findings provided by careful analytical research have been evaluated."

Indeed, a review of the literature does not reveal any controlled research studies on continuous cementum formation but just observations and opinions. We, therefore, decide to set up an investigation to provide data that might answer these questions: (1) Is continuous cementum formation a reality? (2) What is the magnitude of cementum apposition? (3) Is its rate of growth, or continuous growth, the same from year to year, or does it vary or slow down with age? Moreover, our intention was to provide data that could also be subjected to statistical analysis.

MATERIALS AND METHODS

Two hundred and thirty-three single-rooted teeth were used in this study. These were teeth from people ranging in age from 11 to 76 years. The only criterion for selection was the status of the supporting tissue of the teeth—that the roentgenograms and clinical examination showed no pocketing or bone loss around these teeth, so that the supporting tissues of these teeth

This study was started under U. S. Army Contract DA-49-007-MD-623 at the University of Minnesota and was completed under Army Contract DA-49-007-MD-857 at the Eastman Dental Dispensary.

Received for publication March 12, 1958; revised by author July 17, 1958.

could be called "healthy." The teeth were decalcified, embedded in celloidin, and sectioned horizontally from the apex to the cementoenamel junction. The sections were cut at 80 microns, stained with hematoxylin and eosin, and mounted serially. Table I shows the distribution of these teeth according to age and type of tooth. By using an overhead projector microscope, the sections were magnified 25×. The outlines of the cementum and dentin of the enlarged sections were then traced on file-folder paper.* The paper area representing the 25× cementum enlargement was cut out and weighed on an analytical balance. The inside and outside edges of the cementum tracing were measured by the use of a map measurer (in centimeters). To determine cementum thickness from the weight and length measurements the following equation† was used: $\frac{a \times 0.71}{b \times 0.032} =$ cementum thickness.

TABLE I

DISTRIBUTION OF TEETH USED IN STUDY

AGE	TOOTH TYPE							TOTALS
	UPPER CANINES	LOWER CANINES	UPPER LATERAL INCISORS	LOWER LATERAL INCISORS	UPPER CENTRAL INCISORS	LOWER CENTRAL INCISORS	LOWER PRE-MOLARS	
11-20	5	9	7	7	9	10	7	54
21-30	5	–	10	–	19	–	–	34
31-40	9	5	8	–	16	–	–	38
41-50	10	7	9	–	10	1	–	37
51-76	10	14	6	7	14	9	10	70
Totals	39	35	40	14	68	20	17	233

For the first few teeth these measurements were made for every section individually in order to obtain an average cementum thickness for the whole root.‡ However, it was found that this was not necessary. If we obtained measurements for every twentieth section only, then we could obtain a figure for an area of a trapezoid and use the formula $\frac{(y_1 + y_2)\ L}{2}$. An example of such a trapezoid can be seen in Fig. 1 which also shows how, by this method, the volume estimate was obtained for the "total root" from the individual area estimates.

From our drawings we also chose individual areas for determination of cementum thickness: (1) the apical area,§ (2) the area of the cemento-

*Difficulties encountered in distinguishing the cementum-dentin borders, due to the thickness of the sections, were resolved by using a microscope with different magnification up to 400×.

†"a" is the weight, in grams, of the paper cut-out. 0.71 is the factor which converts the weight in grams of the cut-out to the actual cementum volume in cubic millimeters. This factor is obtained as follows: 55.4 × 1/625 ×8/1,000 × 1,000 = 0.71. (The factor 55.4 converts weight, in grams, of the cut-outs, to area, in square centimeters, and is obtained by dividing a known area of the file-folder paper by its weight; 1/625 converts the enlarged area measurement to actual cementum area; multiplying by 8/1,000, the thickness of the sections in centimeters, gives the volume of cementum in cubic centimeters; and multiplying by 1,000 changes cubic centimeters to cubic millimeters.)

"b" is the average of the inside and outside linear measurements of the cementum tracing, in centimeters. 0.032 is the factor which converts the linear measurement of "b" to the actual area measurement in square millimeters. It is obtained as follows: 1/25 × 8/1,000 × 100 = 0.032 (1/25 to reduce the enlargement; 8/1,000 thickness of sections in centimeters, to get area measurement; 100 to convert from square centimeters to square millimeters).

‡Referred to henceforth as "total root."

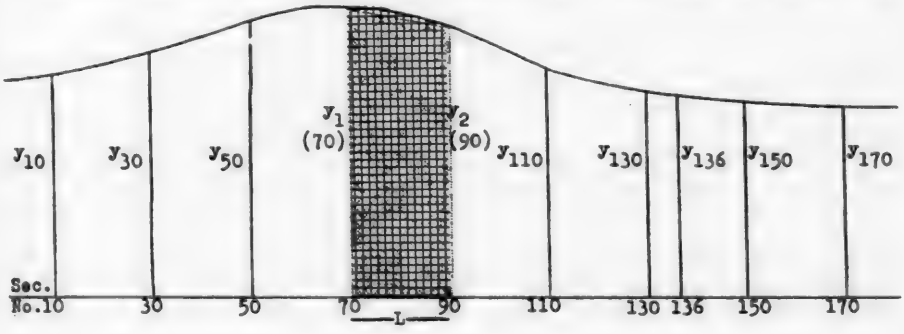
§Referred to henceforth as "apex." This comprises the first 10 sections from the apex (apical 0.8 mm. of root).

enamel junction,* and (3) the middle section of the root.† The average cementum thicknesses for the "total root" and these 3 areas were then tabulated according to age and type of tooth measured. These data were also assembled for statistical analysis.

TABLE II

AVERAGE CEMENTUM THICKNESSES (IN MILLIMETERS)

AGE OF GROUP	TOTAL ROOT	APEX	MIDDLE	CROWN END
11-20	.076 ± .003	.189 ± .011	.072 ± .005	.054 ± .002
21-30	.111 ± .005	.283 ± .017	.115 ± .006	.082 ± .005
31-40	.137 ± .003	.352 ± .006	.161 ± .006	.100 ± .003
41-50	.151 ± .007	.434 ± .028	.157 ± .009	.108 ± .008
51-76	.215 ± .009	.585 ± .034	.238 ± .011	.128 ± .004



Shaded area with sides "y_1" and "y_2" and base "L" shows one of the trapezoids for which formula $\frac{(y_1 + y_2)\,L}{2}$ is used.

The first section in which the cemento-enamel junction appeared in this particular instance was 136.

Fig. 1.—Approximations of areas by trapezoids (linear interpolation).

RESULTS

The cementum thickness for the 54 teeth of the group under 20 years of age averaged 0.076 mm. for the "total root" while it averaged 0.215 mm. for the 70 teeth of the age group from 51 to 76 years. In other words, in the teeth studied the cementum thickness about tripled over this age span. Table II, which contains these figures, also shows that cementum thickness increased for each of the 3 areas studied—the "apex," "middle," and "crown end." It is also apparent that the increase at the "crown end" was much less than at the "apex" while the "middle" was very similar in its increase to the "total root."

*Referred to henceforth as "crown end." This comprises the first section in which the cementoenamel junction appeared (usually on the buccal or lingual side) through the last section showing any cementum.

†Referred to henceforth as "middle." This is the section which is equidistant between the "apex" and the "crown end."

TABLE III

STATISTICAL ANALYSIS OF MEASUREMENTS ON VARIOUS TOOTH TYPES

TOOTH TYPE	EQUATION: $Y = b_0 + b_1$ (AGE)							
	TOTAL ROOT		APEX		MIDDLE		CROWN END	
	$b_0 \cdot 10^4$	$b_1 \cdot 10^5$	$b_0 \cdot 10^4$	$b_1 \cdot 10^5$	$b_0 \cdot 10^4$	$b_1 \cdot 10^5$	$b_0 \cdot 10^4$	$b_1 \cdot 10^5$
Upper canines	854 ± 216	404 ± 52	1062 ± 733	816 ± 176	75 ± 319	494 ± 77	352 ± 179	165 ± 43
Lower canines	159 ± 215	318 ± 46	–407 ± 730	1157 ± 156	–10 ± 318	344 ± 68	251 ± 178	206 ± 38
Upper lateral incisors	439 ± 194	212 ± 51	1601 ± 658	603 ± 170	266 ± 286	259 ± 65	546 ± 160	106 ± 42
Lower lateral incisors	320 ± 251	193 ± 55	1211 ± 907	227 ± 197	238 ± 384	221 ± 83	401 ± 195	101 ± 42
Upper central incisors	101 ± 151	360 ± 38	162 ± 546	872 ± 138	–63 ± 231	463 ± 59	383 ± 117	144 ± 30
Lower central incisors	338 ± 212	240 ± 49	374 ± 763	753 ± 175	298 ± 323	287 ± 74	341 ± 164	145 ± 38
Lower premolars	248 ± 263	396 ± 59	1868 ± 949	1538 ± 199	144 ± 388	413 ± 87	136 ± 217	234 ± 49
For all teeth	325 ± 78	307 ± 18	717 ± 272	859 ± 64	110 ± 116	359 ± 27	275 ± 60	154 ± 15

If one looks at the different tooth types, one can see that the described increase in cementum thickness for all teeth also held true for each individual tooth category.

STATISTICAL ANALYSIS

For statistical purposes a multiple regression equation involving age up to the third power was used. Such an equation would show the significance

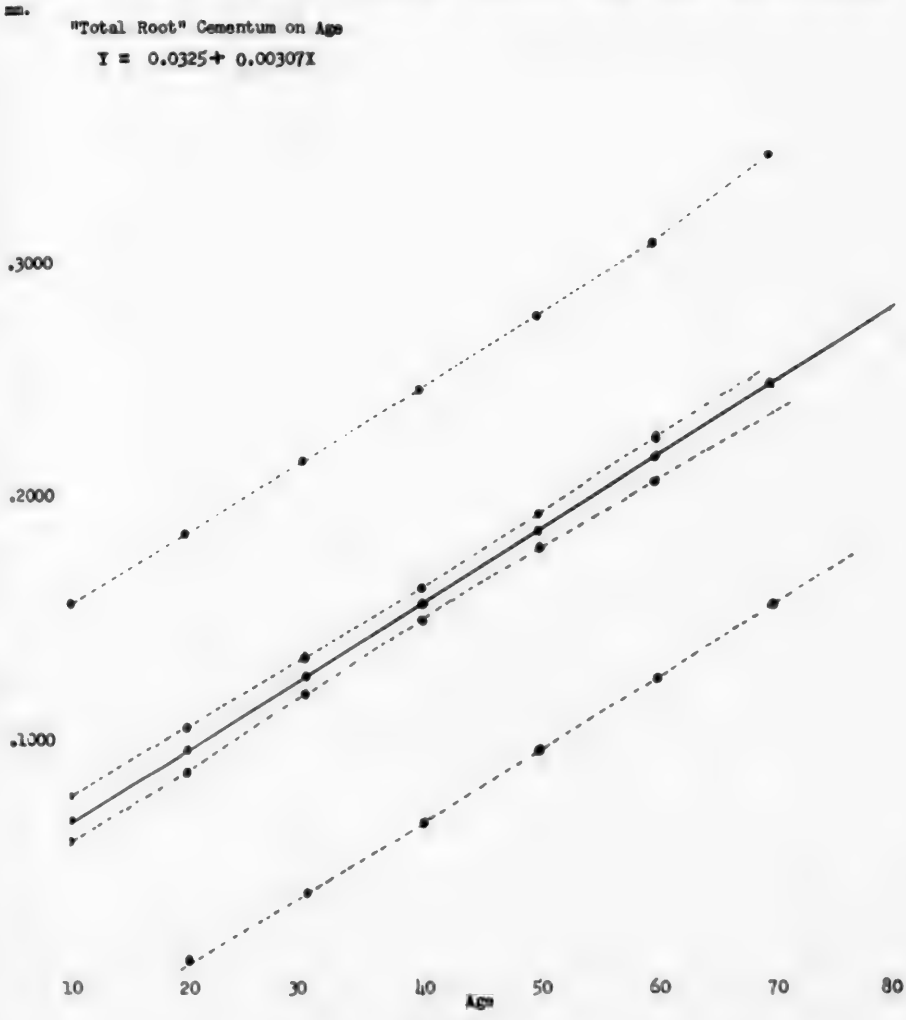


Fig. 2.

of the relationship of cementum thickness to age. This equation was fitted to the data for each of the cementum variables—"total root," "apex," "middle," and "crown end." It was also made for each of the tooth categories—upper canines, lower canines, upper lateral incisors, lower lateral incisors, upper central incisors, lower central incisors, and lower premolars. In all

cases the constant term and the linear regression on age accounted for a very large proportion of the total variability. In other words, the equation $Y = b_0 + b_1$ (age) was sufficient to account for the variability in each of the dependent variables. The values "b_0" and "b_1" and standard deviations were calculated and are presented in Table III. From these standard deviations it

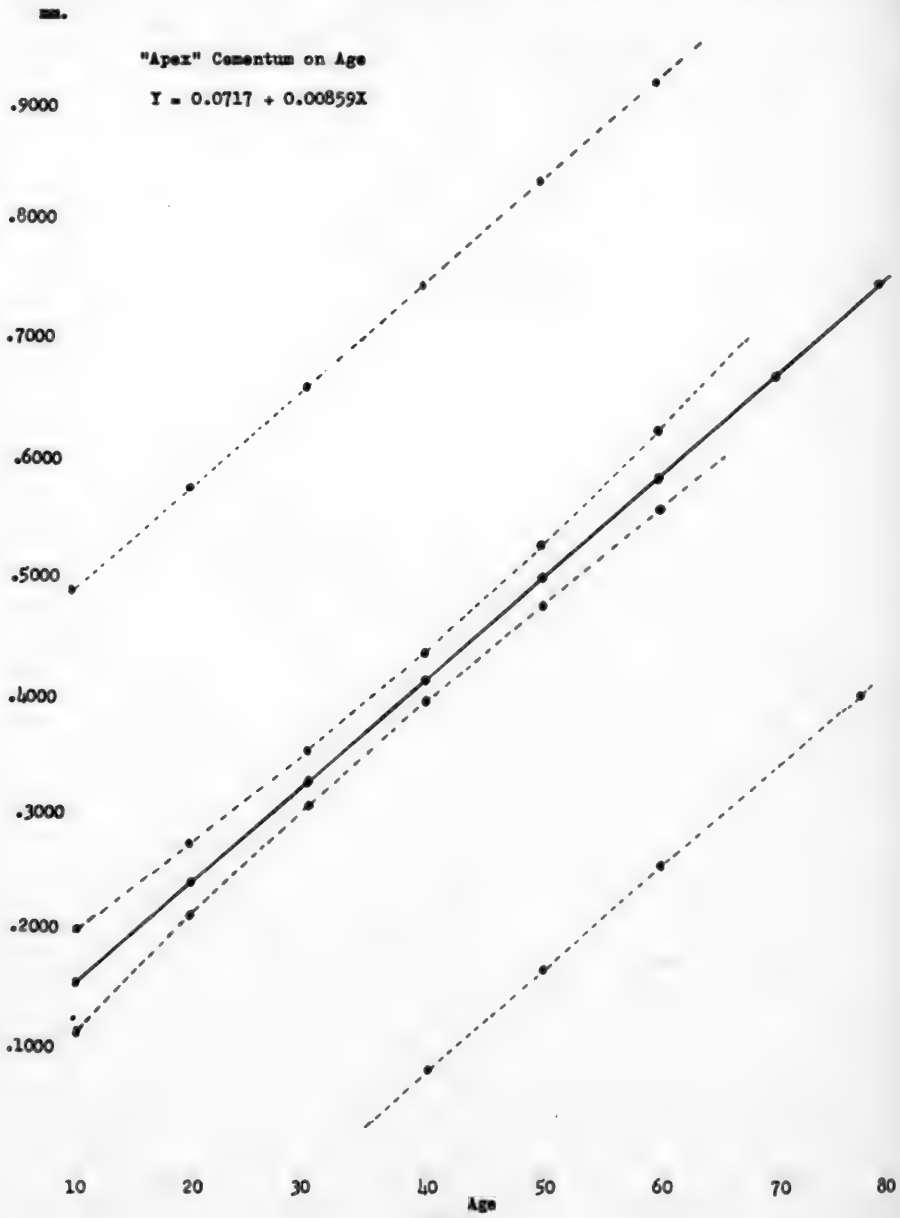


Fig. 3.

is apparent that the values "b_0" and "b_1" do not differ significantly between the different tooth types for either "total root," "apex," "middle," or "crown end" cementum thickness. The properly weighted "averages" are given at the bottom of the table for all teeth studied.

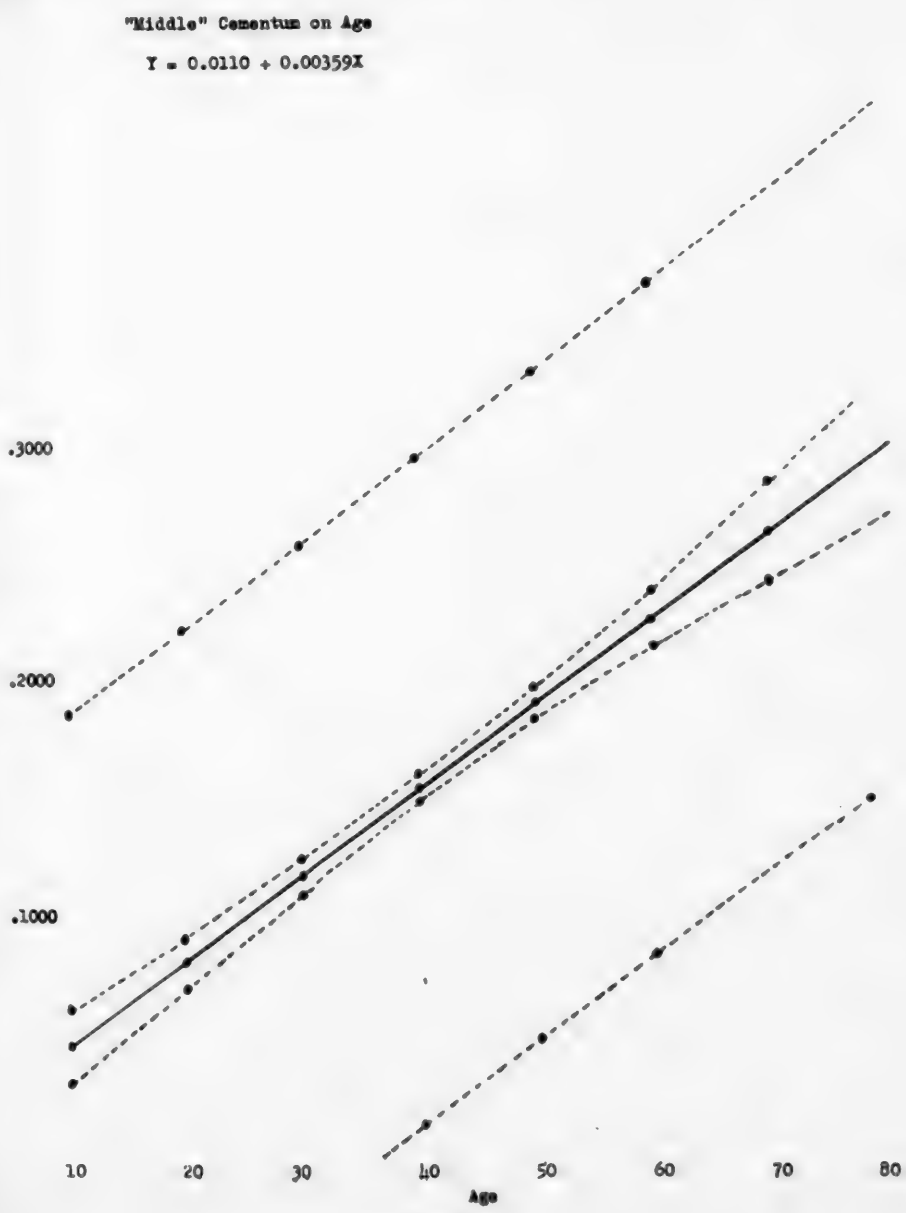


Fig. 4.

The equation for the cementum thickness of the "total root" variable is $Y = 0.0325 + 0.00307X$ where "X" is the age in years. Graphs of the equation for each variable are presented in Figs. 2-5. They show an approximate 95 per cent confidence interval ($p = 0.95$) for the average "Y" for a given age (inside lines) and the individual "Y" (outside lines). From the

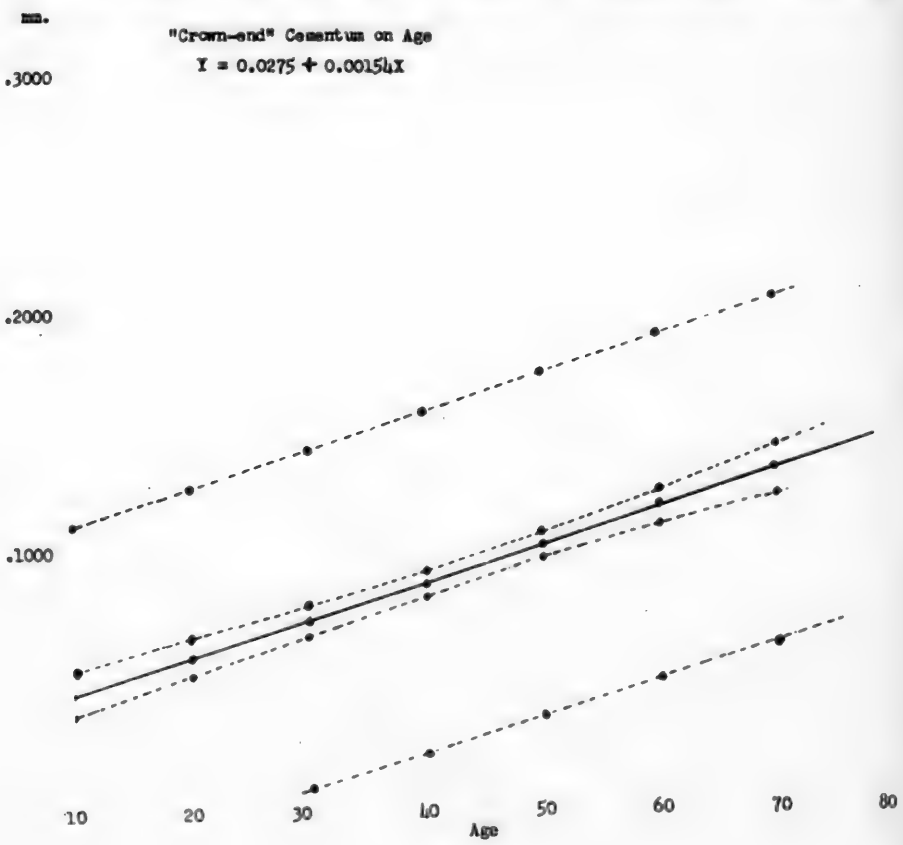


Fig. 5.

data it is also possible to obtain pairwise correlations between the variables. Such a correlation is given for the upper central incisors in Table IV. All of the correlations are significant. As already stated, the measurements for the

TABLE IV

PAIRWISE CORRELATIONS BETWEEN VARIABLES
(FOR UPPER INCISORS)

	TOTAL ROOT	CROWN END	MIDDLE
Crown end	0.695	–	–
Middle	0.945	0.635	–
Apex	0.827	0.527	0.745

"middle" section are most closely associated with the "total root" averages, and least with those of the "crown end." The same order of relationship is apparent for the other tooth types studied.

DISCUSSION

In this series of teeth, supported prior to extraction by healthy periodontal tissues, apposition of cementum seems to have been a continuous process throughout the age span studied, with thickness of the tissue increasing in approximately straight-line relationship with age. This straight-line relationship was surprising in view of the fact that growth processes usually decrease with advancing age. It was interesting to note that the steepness of the line varied considerably with tooth type. The average thicknesses found for the "total root" cementum for all teeth studied (see Fig. 2)—0.095 mm. at age 20 years, 0.125 mm. at age 30 years, 0.155 mm. at age 40 years, 0.185 mm. at age 50 years, and 0.215 mm. at age 60 years—are well within keeping with the data previously reported in the literature. The information presented in the tables and graphs may be useful as a baseline for studies on the cementum thickness of roots from periodontally diseased teeth and from teeth in hypo- and hyperfunction.

SUMMARY AND CONCLUSIONS

Determinations of cementum thickness on 233 single-rooted teeth with healthy supporting tissues showed a straight-line relationship between age and cementum thickness. The thickness of cementum was approximately tripled between ages of 11 and 76 years. This rate was not the same for every area of the root. It was less near the cementoenamel junction and more in the apical area.

In the early stages of working out methods for the reported study, the aid of Gregg Stende and Bert J. McCarthy, two dental students at the University of Minnesota, was invaluable, as was also that of Professor John M. H. Olmsted of the University of Minnesota. Without the help of Dr. Henry B. Clark, Jr., also of the University of Minnesota (Chairman, Division of Oral Surgery, School of Dentistry), who collected the teeth and data pertaining to them, this study would not have been possible.

For the statistical analysis we are indebted to Dr. Arthur M. Dutton of the University of Rochester.

REFERENCES

1. Denton, G. B.: The Discovery of Cementum (personal communication).
2. Magitot, M. E.: Treatise on Dental Caries, Boston, 1878, Houghton, Osgood & Co.
3. Black, G. V.: A Study of the Histological Characters of the Periosteum and Peridontal Membrane, Chicago, 1887, W. T. Keener.
4. Broomell, I. N.: The Histology of Cementum, *D. Cosmos* **XL**: 697, 1898.
5. Stillson, W. C.: A Study of Cementum, *Dental Summary* **37**: 30, 1917.
6. Gottlieb, B.: Die diffuse Atrophie des Alveolarknochens, *Ztschr. f. Stomatol.* **21**: 195, 1923.
7. Kronfeld, R.: The Biology of Cementum, *J. A. D. A.* **25**: 1451, 1938.
8. Weinmann, J., and Sicher, H.: Bone and Bones: Fundamentals of Bone Biology, St. Louis, 1955, The C. V. Mosby Co., p. 119.
9. Gottlieb, B., and Orban, B.: Zahnfleish-Entzuendung und Zahnlockerung, Berlin, 1933, Berlinische Verlaganstalt, pp. 102, 74, and 85, etc.

10. Gottlieb, B.: Biology of Cementum, *J. Periodont.* **13**: 13, 1942.
11. Gottlieb, B.: Histological Consideration of the Supporting Tissues of the Teeth, *J. A. D. A.* **30**: 1872, 1943.
12. Häupel, K.: Paradentitis Marginalis, Oslo, 1926, Fabritius and Sønner.
13. Bauer, W.: Die Apposition und Resorptionsvorgänge an Knochen und Zähnen, *Ztschr. f. Stomatol.* **23**: 185, 1925.
14. Kronfeld, R.: Zementhyperplasien an nicht funktionierenden Zähnen, *Ztschr. f. Stomatol.* **25**: 1218, 1927.
15. Kellner, E.: Histologische Befunde an antagonielosen Zähnen, *Ztschr. f. Stomatol.* **26**: 271, 1928.
16. Bauer, W., and Lang, F. J.: Über das Wandern der Zähne, *Vrtljschr. f. Zahnheilkunde* **44**: 321, 1928.
17. Gottlieb, B., and Orban, B.: Die Veränderung der Gewebe bei übermässiger Beanspruchung der Zähne, Leipzig, 1931, Georg Thieme, pp. 204-205.
18. Kellner, E.: Das Verhältnis der Zement- und Periodontalbreiten zur funktionellen Beanspruchung der Zähne, *Ztschr. f. Stomatol.* **29**: 44, 1931.
19. Gottlieb, B., and Orban, B.: Biology and Pathology of the Tooth and Its Supporting Mechanism, New York, 1938, Macmillan Co., pp. 80-83.
20. Geppert, E.-G., and Müller, K.-H.: Die Wurzelzementapposition als messbarer Ausdruck der Kaudruckbelastung des Zahnes, *Deutsche Zahn-, Mund-, und Kieferheilkunde* **15**: 30, 1951.
21. Wiebusch, F. B.: Periodontal Research: Problems Related to Practice, *J. A. D. A.* **55**: 612, 1957.

CHEMICAL AND PHYSICAL STUDIES OF ENAMEL FROM HUMAN TEETH

II. Specific Gravity, Nitrogen Content, and Hardness Rating of Discolored Enamel

B. R. BHUSSRY*

Department of Anatomy and Division of Dental Research, University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.

AREAS of brown discoloration are often present near the contact point on the proximal surfaces of older teeth. Klebs[1] referred to these areas as "brown marks," Miller[2] called them "decay marks," while both Bibby[3] and Hodson[4] used the term "areas of consolidation." Turesky[5] observed that brown spots were more prevalent in molars and bicuspids, and that there was a positive relationship between the incidence of brown spots and increasing age.

Several studies have dealt with the nature and significance of "brown spot" areas. Miller[2] suggested that the change resulted from a "tanning process." Beust's[6] observations on decalcified sections of similar areas indicated that they are composed of organic material. Hardwick and Manley,[7] and Hodson[4] supported Bibby's[3] idea that brown spots are formed by an organic replacement of the inorganic material lost through decalcification occurring in the mouth.

The purpose of the present study was to characterize these areas of brown discoloration by using histologic, physical, and chemical technics.

MATERIALS AND METHODS

The procedures for selection of teeth, history recording, fixation, and preparation of ground sections have already been discussed in a previous publication.[8] Only the special technics used in this study will be described below.

Selection of Teeth.—Teeth used in this study showed brown spots around the contact point on their proximal surface (Fig. 1). The lesions appeared as dull or shiny areas, usually 1 to 3 mm. in size, and maintained the smoothness of the enamel surface.

Based on a thesis submitted in partial fulfillment of the degree of Doctor of Philosophy, Department of Anatomy, University of Rochester, June, 1956.

This investigation was supported by the Medical Research and Development Board, Office of the Surgeon-General, Department of the Army, under Contract No. DA-49-007-MD 556.

Received for publication March 12, 1958; revised by author July 21, 1958.

*Present address: Department of Anatomy, Georgetown University School of Medicine and Dentistry, Washington 7, D. C.

Polishing and Grinding of Ground Sections.—Plano-parallel ground sections were prepared in order to avoid incorrect interpretation of microscopic observations due to differences in the thickness of various areas of the section. This required an accurate grinding technic. The best results were obtained by using a National Bureau of Standards grinding machine.[9] The sections were ground to an average thickness of 75 to 100 μ without damage to the enamel surface.

Fluorescence Studies.—This work was done at the National Bureau of Standards in cooperation with Dr. A. F. Forziati and G. Dickson who have described the technic.[9]

Fig. 1.—Tooth showing brown spot area.

Decalcification for the Preparation of Paraffin Sections.—The apparatus used (Fig. 2) consisted of a beaker (*A*) with a side arm and a rubber stopper (*B*) with 3 holes each containing a glass tube, 3 mm. in diameter. A hook at the end of one of these tubes carried the celloidin-coated specimen. The other tubes were connected to a compressed air supply and to a reservoir bottle (*D*), respectively. The flow of liquid and air was regulated. Excess fluid in the beaker was drained through the side arm into waste bottles (*E*). An advantage of this apparatus is that handling of the specimen is reduced to a minimum. This is important since the organic material of enamel is very fragile.

The tooth to be decalcified was split sagittally and the dentin from the crown portion of each half was removed by means of steel burrs. After drilling a hole in the root portion, the specimen was coated with a layer of thin celloidin to prevent loss of the fragile organic matrix of enamel during decalcification. The specimen was then suspended in the beaker containing 1 per cent Versene solution at pH 7.5. The flow of air through the solution was adjusted to keep the liquid in gentle motion. Decalcification by this procedure was slow, requiring about 3 weeks for an average specimen.

Upon complete decalcification the specimen was washed with distilled water circulated slowly through the system. The same procedure was repeated for dehydration by using increasing concentrations of alcohols and for clearing by using chloroform. The specimen was then removed from the beaker to the oven where it was embedded in paraffin. Sections were cut at 8 μ thickness, mounted on slides, stained with conventional connective tissue stains, and examined under the microscope.

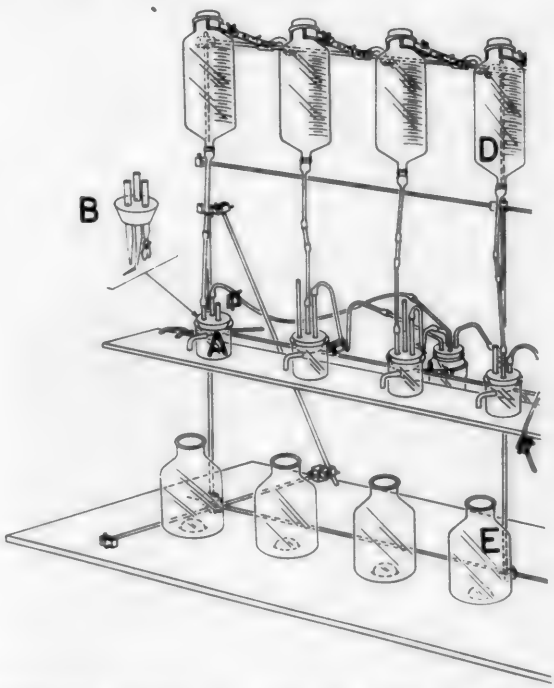


Fig. 2.—Decalcification apparatus. *A*, Beaker with side arm. *B*, Rubber stopper with holes. *D*, Reservoir bottle. *E*, Waste bottle.

Collection of Altered Enamel Samples.—The technic used for collection of altered enamel samples was basically the same as the one described in a previous publication.[8] To avoid incorporation of adjacent sound enamel in the brown spot samples, the desired area was microdissected by directing the jet of abrasive around the altered enamel area in the tooth slab. In some instances, the amount of brown spot sample obtained from individual teeth was not sufficient for analysis, necessitating the pooling of some samples.

Physical and Chemical Determinations.—The apparatus described by Melon and Dallemagne[9] was used for micropycnometric density measurements. The semi-micro-Kjeldahl procedure was employed for nitrogen determinations. A Tukon hardness tester[10] was used for hardness measurements.

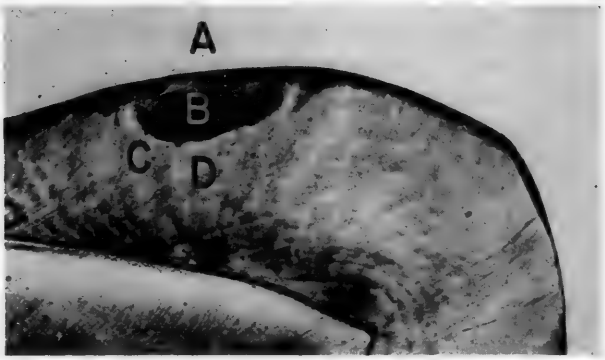


Fig. 3.—Unstained ground section through a brown spot area of enamel. Posteruptive age, 13 years. (Orig. mag. × 25.)

A, Surface contour. *B*, Center of the lesion showing rod sheaths and incremental line. *C*, Zone of consolidation. *D*, Normal enamel.

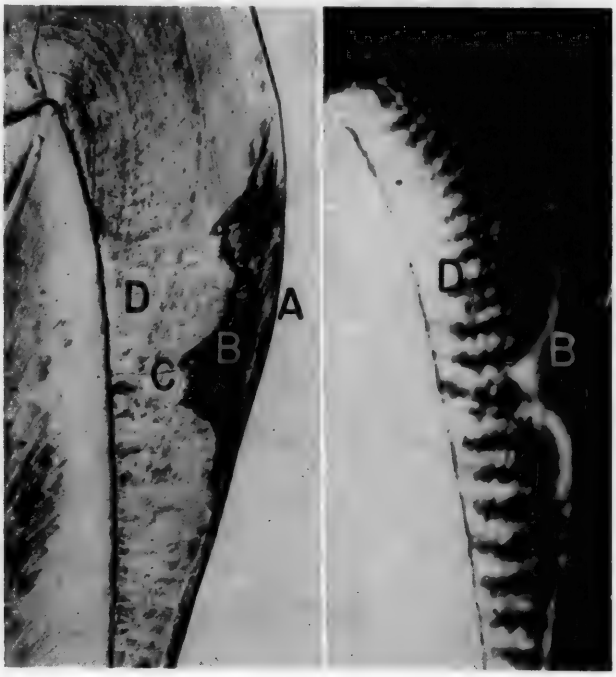


Fig. 4. Fig. 5.

Fig. 4.—Ground section through a brown spot area. Posteruptive age, 45 years. (Orig. mag. × 25.)

Fig. 5.—Fluorophotomicrograph of the brown spot area in Fig. 5. Note the absence of fluorescence along the enamel surfaces. (Orig. mag. × 25.)

RESULTS

Microscopic Study.—

A. *Ground sections:* Examinations by transmitted light under low power (× 100) were made on 36 ground sections showing brown spot areas. The extension of the lesion in the enamel varied in every section. The histologic structure was, however, similar in all specimens. Fig. 3 represents an unstained ground section through a brown spot area of low intensity in a tooth from a patient aged 23. The surface contour (*A*) in the area of altered enamel is intact. The rod sheaths, the incremental lines, and cross striations (*B*) in this area are more prominent than in the adjacent sound enamel.

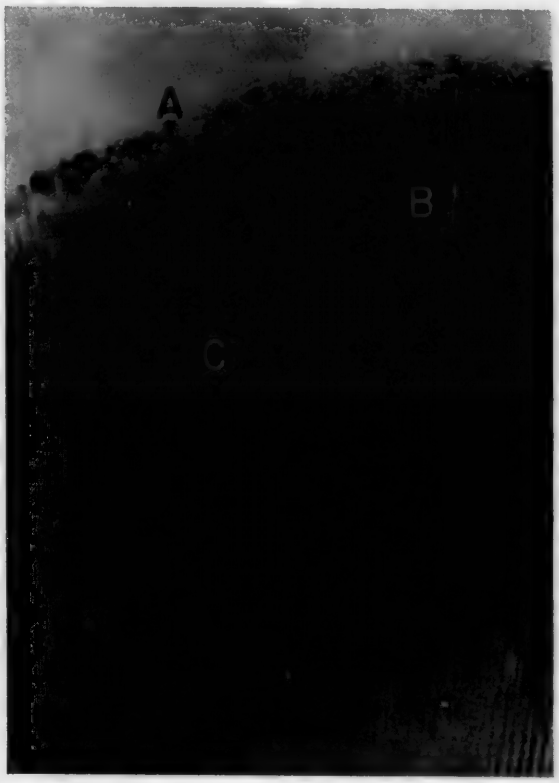


Fig. 6.—Unstained decalcified enamel section showing brown spot area. Posteruptive age, 20 years. Note the continuation of rod sheaths between the brown area (*B*) and sound enamel (*D*). (Orig. mag. × 210.)

The brown spot seems to be demarcated by a zone of consolidation (*C*). Examination of this area under higher magnification shows that the rod sheaths and incremental lines in the lesion are continuous with those in adjacent sound enamel. An aggregation of brown pigmented material in the center of the lesion partly obscures the structure of the rod sheaths. The organic framework in the lesion, however, maintains its structure and orientation.

Fig. 4 is a ground section through a brown spot area of a tooth from a patient aged 55. The histologic picture is the same as described for Fig. 3, except that there is an increased amount of pigmented material in the center of the lesion. When examined under ultraviolet light (Fig. 5), the main part of the lesion (*B*) does not fluoresce and the rod sheaths and incremental lines are not visible. The zone of consolidation (*C*), however, emits a greater intensity of fluorescence than the normal enamel.

Twenty-five ground sections showing brown spot areas were subjected to acid decalcification under the microscope.[11] Decalcification changes were first

Fig. 7.—Paraffin section through the brown spot area of a tooth. Posteruptive age, 55 years. Stained with hematoxylin and eosin. (Orig. mag. × 917.)

A, Microorganisms and debris. *B*, Altered enamel zone. *C*, Normal enamel. *D*, Altered enamel with well-defined prism structure. *E*, Pathways in enamel.

noticeable in the sound enamel around the lesion (Fig. 6). The enamel rods, incremental lines, and cross striations in this area became more prominent. Whether decalcification took place through the prisms or the interprismatic substance was not clear. The zone of consolidation maintained its original appearance. When decalcification of sound enamel around the lesion was complete more residue was left in the brown spot area than the adjacent sound enamel.

B. *Paraffin sections:* Serial sections were made from 20 decalcified specimens showing brown spot areas. Fig. 7 represents a section (× 917) through such a lesion in a tooth from a 55-year-old patient. Since this tooth was not well cleaned before decalcification, an area of densely packed microorganisms

and debris (*A*) is present on the surface. The deeply stained area under this is the zone of altered enamel (*B*). The surrounding normal enamel (*C*) shows well-defined prism sheaths and cross striations (*D*) demonstrating the integrity of the organic matrix in these areas. Whether the pathways (*E*) running from the surface inward are artefacts or were created during the progress of the lesion is not known.

Hardness Study.—

Fourteen ground sections from teeth showing brown spots were used for this study. Forty-four longitudinal indentations were placed in the brown spot area, 10 in the consolidated zone, and 52 in the adjacent sound enamel.

Fig. 8.—Ground section showing indentations for microhardness measurements. (Orig. mag. × 100.)

A, Indentation in sound enamel. *B*, Indentation in the consolidated zone. *C* and *C'*, Indentations in the brown spot area.

The size and shape of a typical indentation is shown at (*A*) in Fig. 8. For the sake of convenience in manipulation, all serial indentations were placed longitudinally in a single plane from the surface inward and numbered. No difference in hardness could be detected in sound enamel when indentations were placed longitudinally or transversely to the direction of the enamel rods. These determinations are summarized in Table I. It is obvious that the brown spot areas have the lowest hardness number (95), the consolidated zone gives an intermediate figure (142), while the sound enamel is the hardest (252).

Density and Nitrogen Determinations.—

A comparison of the density and nitrogen content of brown spot areas and sound enamel is summarized in Table II. The brown spots were divided

into under 30 and over 30 years posteruptive age groups, since the exact age of the initiation of brown spots could not be ascertained. There was a great deal of variability in the density and nitrogen values of individual brown spots.

It is apparent from these data that the density of brown spot areas is lower than that of sound enamel. The nitrogen content, on the other hand, is higher in these areas both on weight and volume basis.

TABLE I
COMPARATIVE HARDNESS OF BROWN SPOTS AND SOUND ENAMEL
(SUMMARY OF RESULTS)

INDENTATION	NO. OF INDENTATIONS	AVERAGE KNOOP HARDNESS NO.
Brown Spot		
Longitudinal	44	95
Consolidated Zone		
Longitudinal	10	142
Sound Enamel		
Longitudinal	47	252
Transverse	5	250
Longitudinal and transverse	52	252

TABLE II
COMPARISON OF BROWN SPOTS AND SOUND ENAMEL

	DENSITY—GM./CM.^{3}		N_2 CONTENT—MG./GM.		N_2 CONTENT—MG./CM.^{3}	
	NO. OF TEETH	MEAN S.E.*	NO. OF TEETH	MEAN S.E.*	NO. OF TEETH	MEAN S.E.*
Under 30 yr.						
Brown spots	48	2.50 ± 0.04	62	0.87 ± 0.07	48	2.00 ± 0.22
Sound enamel	238	2.82 ± 0.02	244	0.49 ± 0.02	234	1.45 ± 0.02
Over 30 yr.						
Brown spots	7	2.39 ± 0.10	15	1.44 ± 0.15	7	3.66 ± 0.58
Sound enamel	48	2.77 ± 0.04	62	0.66 ± 0.04	34	1.93 ± 0.16

*The standard errors for brown spot analyses are based on the within group standard deviations (pooled estimates), which are as follows: Density, 0.29; Nitrogen content mg./Gm., 0.57; Nitrogen content per cm.^{3}, 1.54.

DISCUSSION

The data support the general conclusion that there is a definite increase in the organic content of the brown spot areas. Histologically, it appears that the pigmented material is present between the rod sheaths and also in the rod itself. The demonstration that the pigmented material is acid-resistant suggests that the material in these areas is composed largely of organic matter. Furthermore, the increased residue on acid decalcification may account for the higher nitrogen content of the brown spots. The lower density and decreased hardness of these areas suggest that a concomitant reduction in mineral content may occur, although direct chemical evidence for this has not yet been obtained. The origin and nature of the organic material in these areas and the manner in which it may replace some of the inorganic constituents is still unknown.

SUMMARY

1. Microscopic study of brown spots suggests an alteration in the organic material in these areas. The presence of increased residue on decalcification of brown spots indicates an increase in the organic content of the lesion.

2. The organic content of these areas is higher than that of sound enamel according to the nitrogen analysis.

3. The finding that the brown spots have a decreased hardness and lower density than the sound enamel suggests a loss of inorganic material in these areas.

4. The nature of these changes is not fully understood, but it seems likely that the increased organic content is of extraneous origin.

REFERENCES

1. Klebs, E.: Beitrage zur Kenntnisse der pathogenen Schistomyceten, *Arch. Exper. Path.* **5**: 350, 1876.
2. Miller, W. D.: Microorganisms of the Human Mouth, Philadelphia, 1890, S. S. White Dental Mfg. Co.
3. Bibby, B. G.: Organic Structure of Dental Enamel as a Passive Defense Against Caries, *J. D. Res.* **12**: 99, 1932.
4. Hodson, J. J.: A Histopathological Study of the Bacterial Plaque in Relation to the Destruction of Enamel, Dentin, and Bone With Special References to Dental Caries, *Proc. Roy. Soc. Med.* **48**: 641, 1955.
5. Turesky, S. S.: Brown Spots, *Tufts Dental Outlook* 2: 15, 1941.
6. Beust, T. B.: Microorganisms and Caries, *J. A. D. A.* **17**: 1536, 1930.
7. Hardwick, G. L., and Manley, E. B.: Caries of the Enamel. II. Acidogenic Caries, *Brit. D. J.* **92**: 225, 1952.
8. Bhussry, B. R.: Chemical and Physical Studies of Enamel From Human Teeth. I. Specific Gravity and Nitrogen Content of Enamel From Different Surfaces, *J. D. Res.* **37**: 832, 1958.
9. Dickson, G., and Forziati, A. F.: Fluorescence of Teeth as a Means of Investigation of Their Structure, *J. A. D. A.* **45**: 661, 1952.
10. Lysaght, V. E.: Indentation Hardness Testing, New York, 1949, Reinhold Publishing Co., pp. 191.
11. Bhussry, B. R., and Bibby, B. G.: Surface Changes in Enamel, *J. D. Res.* **36**: 409, 1957.

CHEMICAL AND PHYSICAL STUDIES OF ENAMEL FROM HUMAN TEETH

III. Specific Gravity, Nitrogen Content, and Histologic Characteristics of Opaque White Enamel

B. R. BHUSSRY*

Department of Anatomy and Division of Dental Research, University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.

ON THE BASIS of clinical location and gross appearance, two types of white opaque enamel may be distinguished: (1) white opaque areas of enamel on the approximal surface of young teeth, commonly referred to as white spots or early carious lesions. These are regarded as the result of acid action on enamel, (2) white enamel areas, diffuse in nature, and present as large irregularly distributed bands in enamel, commonly referred to as hypocalcified developmentally defective areas.

As a result of histologic observations and the increased stainability of these areas, the belief has been expressed that this altered enamel has a higher organic content than sound enamel. Hardwick and Manley,[1] and Hodson[2] claim that the increase in organic material of such opaque areas is due to the adsorption of material from the saliva. Biochemical studies of white opaque enamel by Coolidge,[3] however, revealed no significant difference in the nitrogen content of sound and altered enamel. Stack[4] on the other hand, showed that chalky enamel contains 3 to 4 times as much organic material as the adjacent sound enamel. There have apparently been no attempts to determine the density of such areas.

The present investigation was undertaken to characterize the two types of opaque enamel as to histologic structure, density, and nitrogen content and to compare these findings with corresponding data on sound enamel.

MATERIAL AND METHODS

The procedure for selection of teeth and recording of their history, the fixation and preparation of ground sections, and the special technics (collection of enamel samples; density and nitrogen determinations) used for studying the altered enamel areas have been reported in previous publications (Bhussry[5, 6]). It should be mentioned, however, that on the basis of clinical

Based on a thesis submitted in partial fulfillment of the degree Doctor of Philosophy, Department of Anatomy, University of Rochester, June, 1956.

This investigation was supported by the Medical Research and Development Board, Office of the Surgeon-General, Department of the Army, under Contract No. DA-49-007-MD 556.

Received for publication March 12, 1958; revised by author July 21, 1958.

*Present address: Department of Anatomy, Georgetown University School of Medicine and Dentistry, Washington 7, D. C.

Fig. 1.

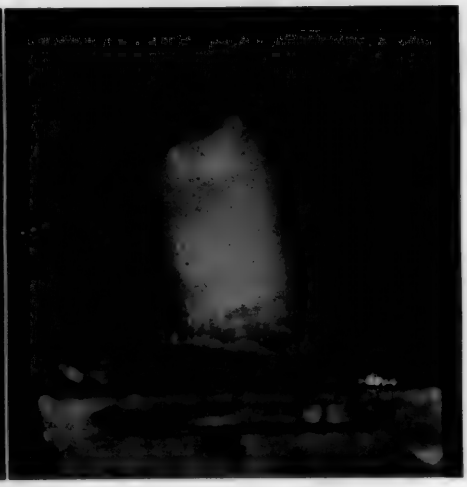


Fig. 2.

Fig. 1.—Photograph of a white spot area.

Fig. 2.—Photograph showing the clinical appearance of a hypocalcified developmentally defective area.

Fig. 3.

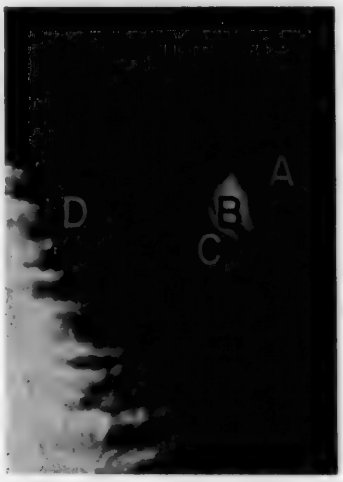


Fig. 4.

Fig. 3.—Ground section through a white spot area of enamel. Posteruptive age, 5 years. (Orig. mag. ×25.)

A, Smooth surface contour. *B*, Center of the lesion showing rod sheaths and incremental lines. *C*, Zone of consolidation. *D*, Normal enamel.

Fig. 4.—Fluorophotomicrograph of the white spot area shown in Fig. 3. (Orig. mag. ×25.) Note the increased fluorescence in the area of the lesion.

location and gross appearance the two types of enamel used in this study were classified as: (1) white spots (Fig. 1) or (2) hypocalcified developmentally defective areas (Fig. 2).

OBSERVATIONS

Microscopic Study.—Histologic observations were made on ground sections (75-100 μ thickness) from 28 teeth with white spots and 16 with developmentally defective areas. The sections were examined by transmitted light under low power (100×) of the microscope.

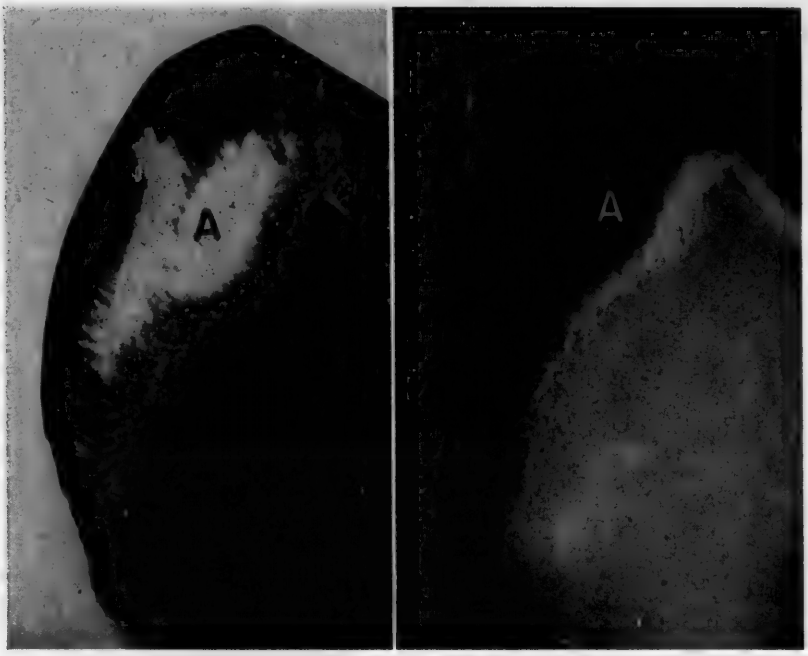


Fig. 5. Fig. 6.

Fig. 5.—Unstained ground section through a hypocalcified developmentally defective area in enamel. Posteruptive age 8 years. (Orig. mag. ×25.)

A, Irregular translucent zone. Note the presence of rod sheaths in this area.

Fig. 6.—Fluorophotomicrograph of the hypocalcified developmentally defective area in Fig. 5. (Orig. mag. ×25.)

All white spot areas examined in ground sections had a number of features in common, which are represented in Fig. 3. All showed smooth surface contour *(A)*, the presence of rod sheaths, incremental lines and cross striations *(B)*, which were more prominent in the area of the lesion as compared to adjacent sound enamel. The center of the lesion showed varying amounts of pigmented material. The structure and orientation of the organic framework was maintained. The extent of penetration of the lesion into the enamel was demarcated by a consolidated zone *(C)*. When the same area was examined by ultraviolet illumination (Fig. 4), the main part of the lesion *(B)* was more

highly fluorescent than the adjacent sound enamel, but the rod sheaths and incremental lines were not visible. The surface contour, although visible, did not fluoresce. The zone of consolidation *(C)* was well marked and emitted a brighter fluorescence than the sound enamel *(D)*.

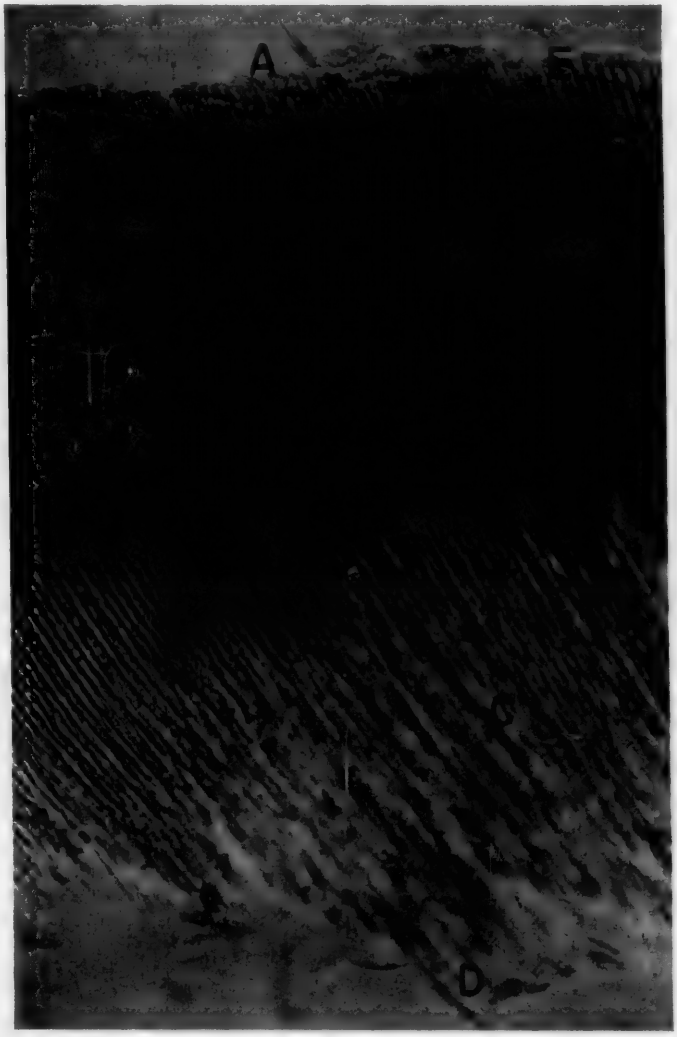


Fig. 7.—Unstained enamel section (partially decalcified) showing a white spot area. (Orig. mag. ×210.)

A, Enamel surface. *B*, Center of the lesion. *B'*, Zone of consolidation. *C*, Normal enamel. *D*, Completely decalcified sound enamel. *E*, Surface showing signs of breaking.

Histologically, the hypocalcified developmentally defective areas appear as diffused irregular translucent zones in enamel (Fig. 5). The structural components were the same as seen in the white spots, but the rod sheaths and

incremental lines were not as prominent and the zone of consolidation was absent. Under ultraviolet light (Fig. 6), the translucent zone did not fluoresce, but the rod sheaths were clearly visible.

Eighteen ground sections showing white spot areas were subjected to controlled decalcification[6] under the microscope. The first visible decalcification changes were observed in the sound enamel around the lesion (Fig. 7). A few hours later the continuation of rod sheaths and incremental lines between the lesion *(B)* and the adjacent sound enamel *(C)* was also noticed. The resistance of white spots to solubility in acids continued for about 23 to 40 hours, after which time, the structural components on the surface began to show signs of breaking down *(D)*. On complete decalcification, the histologic structure in the lesion appeared to be the same as that in the adjacent sound enamel, except that the zone of consolidation *(B')* was still present.

Density and Nitrogen Determinations.—A comparison of the density and nitrogen content of the white spots, hypocalcified developmentally defective areas, and sound enamel is presented in Table I. It is apparent from the data that the density of both types of opaque enamel is lower than that of sound enamel. When the two types of opaque enamel are compared, the white spot areas show a lower density than the hypocalcified developmentally defective areas. With regard to the nitrogen content, it is evident that although there are no differences in the values between the two types of opaque enamel, the values are higher than those for sound enamel both on weight and volume basis.

TABLE I

COMPARISON OF ALTERED AND SOUND ENAMEL

	DENSITY—GM./CM.^{3}			N_2 CONTENT—MG./GM.			N_2 CONTENT—MG./CM.^{3}		
	NO. OF TEETH	MEAN	S.E.*	NO. OF TEETH	MEAN	S.E.*	NO. OF TEETH	MEAN	S.E.*
White spots	19	2.44 ± 0.07		42	0.70 ± 0.04		18	1.97 ± 0.23	
Developmentally defective	26	2.62 ± 0.06		26	0.72 ± 0.08		26	1.94 ± 0.19	
Sound enamel (0-30 yr.)	238	2.82 ± 0.02		244	0.49 ± 0.02		234	1.45 ± 0.06	

*The standard error is based on the within group standard deviations (pooled estimate), which are as follows: Density, 0.29; Nitrogen content per gram, 0.57; Nitrogen content per cubic centimeter, 1.54.

DISCUSSION

The findings on white opaque enamel support the view[4, 6, 7, 8] that there is not only an alteration in structure, but an increase in the organic material in both types of white opaque areas. Although of different origin and clinical location, both have many histologic features in common and both have higher nitrogen content than the adjacent sound enamel.

The observation that the deeper layers of the white spot areas emitted brighter fluorescence under ultraviolet light than the adjacent normal enamel might also be considered evidence of an increase in the organic content.[9] This alteration, corresponding to the lowered density in these areas, is probably due to a reduction in mineral content.

Further support for the idea that there is a reduction of the inorganic material in opaque enamel comes from the hardness studies of Gustafson[10] and Muntz,[11] and the soft x-ray studies of Darling,[12] Hodson,[13] and Guzman.[14] The former reported that the hardness of these areas was less than that of sound enamel. The latter investigators observed that such areas were more readily penetrated by Grenz rays, indicating a loss of x-ray density.

SUMMARY

1. A study has been conducted to characterize the white opaque enamel as to histologic structure, density, and nitrogen content.

2. On the basis of clinical location and gross appearance, two types of opaque enamel were studied: (a) white spot areas indicative of early carious lesions, and (b) hypocalcified developmentally defective areas.

3. Microscopic observation of ground sections from white spot areas and teeth with developmentally defective areas indicated a similarity of the histologic structure in both varieties of white opaque enamel. The main exception was that, in the latter, the zone of consolidation was absent.

4. The density and nitrogen determination of opaque enamel suggest that both types have higher nitrogen content and lower density than the adjacent sound enamel.

REFERENCES

1. Hardwick, J. L., and Manley, E. B.: Caries of the Enamel. II. Acidogenic Caries, *Brit. D. J.* **92**: 225, 1952.
2. Hodson, J. J.: A Histopathological Study of the Bacterial Plaque in Relation to the Destruction of Enamel, Dentin, and Bone With Special Reference to Dental Caries, *Proc. Roy. Soc. Med.* **48**: 641, 1955.
3. Coolidge, T. B.: Nitrogen and Phosphorus of Early Smooth Surface Caries of Enamel, *J. D. Res.* **30**: 97, 1951.
4. Stack, M. V.: The Organic Content of "Chalky" Enamel, *Brit. D. J.* **96**: 73, 1954.
5. Bhussry, B. R.: Chemical and Physical Studies of Enamel From Human Teeth. II. Specific Gravity, Nitrogen Content, and Hardness Rating of Discolored Enamel, *J. D. Res.* **37**: 1045, 1958.
6. Bhussry, B. R., and Bibby, B. G.: Surface Changes in Enamel, *J. D. Res.* **36**: 409, 1957.
7. Malleson, H. C.: The Histology of Enamel Caries, *Brit. D. J.* **46**: 907, 1925.
8. Sullivan, H. R.: Formation of Early Lesions in Dental Enamel, *J. D. Res.* **33**: 218, 1954.
9. Forziati, A. F.: Personal communication, 1956.
10. Gustafson, G., and Kling, O.: Micro-hardness Measurements in Human Dental Enamel, *Sartrych ur Odont. Tidsk.* **1**: 24, 1948.
11. Muntz, M. L., Gilmore, R. W., Caldwell, R. C., and Pigman, W.: Micro-hardness Studies of Intact Surface Enamel. Preprinted Abstracts, 34th General Meeting I. A. D. R., St. Louis, 1956.
12. Crabb, H. S. M., and Darling, A. I.: X-ray Investigations of Human Dental Enamel, *J. D. Res.* **32**: 731, 1953 (Abst.).
13. Hodson, J. J.: Identification of Hypocalcified and Demineralized Areas in Sections of Human Enamel, *Nature* **175**: 261, 1955.
14. Guzman, C.: Micro-radiographic Studies of "White Spots," M.S. Thesis, University of Rochester, N. Y., 1955.

THE MEASUREMENT OF THE ABRASION OF HUMAN TEETH BY DENTIFRICE ABRASIVES: A TEST UTILIZING RADIOACTIVE TEETH

R. J. GRABENSTETTER, R. W. BROGE, F. L. JACKSON, AND A. W. RADIKE

The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio

THE MEASUREMENT of the abrasiveness of dentifrice abrasives toward human teeth has occupied the attention of many workers since the first study by W. D. Miller[1] in 1907. He found, by the hand brushing of freshly extracted teeth mounted in plaster of Paris, that the commonly used dentifrices were capable of producing marked damage to the teeth. This damage was noted clinically as wedge-shaped notches in the cervical region of anterior teeth. Some of the materials tested could abrade enamel in relatively short brushing times. The attention given to such materials has resulted in the use of much less abrasive materials in dentifrices with a consequent decrease in enamel abrasion to the point where it is now of little concern. The abrasion of dentin subsequently received attention, when it was demonstrated by Bunting and Rickert,[2] Head,[3] Carney,[4] and Wright and Fenske[5] that dentin is worn away relatively rapidly by dentifrice abrasives. Calcium carbonate dentifrice abrasives, which did not damage enamel, produced marked wear of cementum and dentin. Since a great many people experience some recession of the gum line, such abrasion is a real problem.

The earlier methods used in measuring the abrasiveness of dentifrice abrasives varied from hand brushing of extracted teeth,[1] which gave qualitative comparisons, to precise measurement of the rate of weight loss of metal blocks held against a rotating surface covered with a slurry of the abrasive under test.[6] Later work by Tainter[7] and Epstein[8] indicated that there is no simple correlation between metal block abrasion and tooth abrasion. The surface flow of metals results in a lower rating for a given abrasive on the basis of metal abrasion than is found in actual tooth abrasion, and varies with the abrasive and the metal. Therefore, it appeared that a reliable method of determining the abrasiveness of dentifrice abrasives toward either dentin or enamel must involve the use of human teeth, preferably freshly extracted, moist teeth suitably protected from bacterial attack during storage. The most satisfactory quantitative methods of evaluating abrasiveness using human teeth involve the use of a brushing machine and the measurement of the depth of a cut produced in the surface of a suitably mounted tooth. The necessity of using moist teeth complicates measurement of weight loss during abrasion.

Received for publication April 4, 1958; revised by authors Aug. 4, 1958.

An early machine was described by Van der Merwe,[9] in which the brush moved perpendicularly to the long axis of the tooth, the tension of the brush being controlled by springs. A later adaptation is described by Tainter and Epstein.[10] The machine used in the present study was of this type and was developed by Manly.[11] The single brush model[11] was enlarged by Manly to the 8 brush model described herein. The depth-of-cut method[11] of comparing abrasives using the cross-brushing machine involves a large variability, a great part of which is inherent in cutting and measuring the depth of a groove in the tooth. In order to circumvent this major source of error and to shorten the procedure, the present method using radioactive human teeth was developed.

METHODS

A. *Description of Method Used.*—

Teeth rendered radioactive by exposure to neutrons, as described below, are mounted in Wood's metal and fastened in place beneath the tooth brushes in the cross-brushing machine (Fig. 1). The pressure of the brush on the tooth is adjusted to 150 Gm., and the tooth and brush are immersed in a tube of a slurry of the abrasive to be tested. The machine is run for suitable increments of brush strokes (usually 1,000). A 2 ml. sample of the slurry is withdrawn with a pipette, deposited in a weighed planchet, dried at 105° C., and weighed. The radioactivity of the dried samples is determined and the amount of tooth structure corresponding to the counts of each sample is calculated in terms of the micrograms of tooth structure abraded per 100 brush strokes.

After every test of an unknown abrasive, a standard abrasive ($CaCO_3$, No. 1) is run on the same teeth under the same conditions. Abrasives are then compared in terms of the "Abrasion Ratio," where

$$\text{Abrasion Ratio} = \frac{\text{Abrasiveness of the unknown in a given run}}{\text{Abrasiveness of a standard, run immediately afterward}}.$$

Comparison with a standard abrasive, run immediately after the unknown, minimizes the variations due to progressive changes in size and shape of the tooth area brushed.

B. *Irradiation of Human Teeth.*—

The teeth were made radioactive by exposure to neutrons in the Oak Ridge Pile, having first been separated into crown (enamel) and root (dentin) portions.

During irradiation, a part of the P^{31} in hydroxyapatite is converted into P^{32} by the following reaction:

$$Ca_{10}(P^{31}O_4)_6(OH)_2 + n^0 \rightarrow Ca_{10}(P^{32}O_4)_6(OH)_2 + \text{gamma radiation.}$$

Calcium, by neutron capture, gives rise to small amounts of isotopes which emit weak beta radiation; oxygen produces beta-gamma emitters of very short half-life, and hydrogen produces stable deuterium. The other materials present are in such small quantities as to be unimportant. Hence, only phosphorus

in the teeth gives rise to sufficiently long-lived radioisotopes in large enough amounts for practical measurements. The detection of P^{32} depends upon counting the relatively high-energy electrons emitted during the decay process. The half-life of P^{32} is 14.3 days.

The teeth are irradiated in a sealed aluminum capsule containing a few milliliters of dilute formaldehyde solution to protect the teeth from bacterial attack before irradiation, as well as to maintain the moisture content. Irradiation is carried on for one week at a neutron flux of $\sim 5 \times 10^{10}$ n°/cm.²/sec.

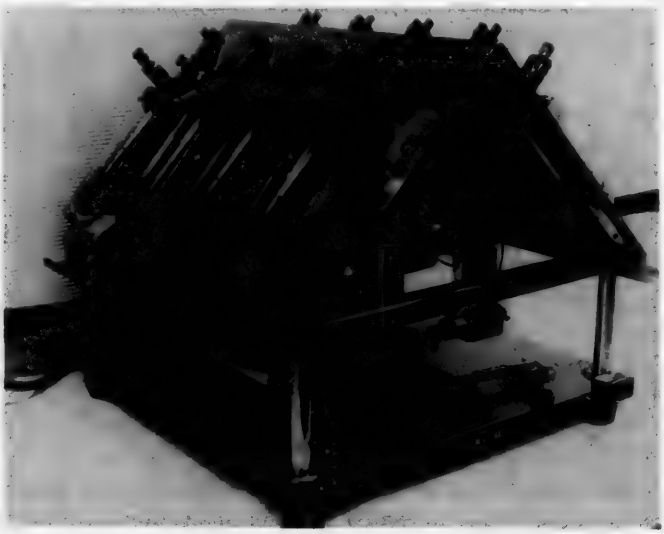


Fig. 1.—Cross-brushing machine.

at a temperature under 40° C. This results in about one millicurie of radioactivity per tooth. Since about 10^{-6} mc. of activity can be determined accurately and each tooth weighs about 1.0 Gm., about 1×10^{-6} Gm. of tooth structure can be determined.

The teeth are unchanged in appearance after irradiation under the conditions specified. Hardness measurements of both enamel and dentin performed with a Tukon Hardness Tester indicate no change within limits of error as shown in Table I.

TABLE I

EFFECT OF NEUTRON IRRADIATION UPON THE HARDNESS OF DENTIN AND ENAMEL

	KNOOP HARDNESS NO. (KG./MM.²)	
	ENAMEL	DENTIN
Before irradiation	343 ± 22	54.2 ± 5.6
After irradiation	368 ± 23	60.4 ± 6.5

Irradiation at < 40° C; neutron flux = $\sim 5 \times 10^{10}$ n°/cm.²/sec.; Tukon Hardness Tester, 100 Gm. load; Knoop diamond indenter; average of 10 teeth, 2 determinations per tooth.

Variations in hardness between different spots on the same tooth are as large as the variations between different teeth, and of the same magnitude as the variation attributable to irradiation.

Theoretical considerations of the irradiation process indicate that the radioactive material (P^{32}) is uniformly distributed throughout the irradiated tooth structure.

C. *Apparatus and Materials.*—

1. *Cross-brushing machine:* The brushing machine (Figs. 1 and 2) is of a design reported previously,[11] but with modifications in the brush-mounting and tooth-mounting methods as shown. Brush tension is adjusted by means of the spring adjustment screws with the aid of a tension gauge* (shown in Fig. 1 just beneath the counter). The number of strokes is counted by means of a Veeder-Root Counter permanently mounted on the machine. The brushes

Fig. 2.—Tooth mounting.

used were of straight-head type with medium-hard nylon bristles modified from a serrated to a straight trim. A stainless steel agitating fin is fastened to the end of the brush, as shown, to ensure adequate mixing. The machine performs about 200 strokes per minute.

The irradiated teeth are mounted in Wood's metal, using the aluminum mold shown in Fig. 4. The cavity in block *A* (Fig. 4) is filled with a plastic support (Melotte Moldine) to hold the tooth. Plates *B* and *C* are then placed in position over the tooth and the cast is made.

The mounted teeth are stored under water to retain moisture and reduce radiation hazard, since the beta-radiation involved is stopped by a relatively thin layer of water or any solid material. The irradiated teeth should always be handled by forceps and kept at least 10 to 20 cm. from the hands. Permissible hand exposures at various working distances are shown in Fig. 3.

*Manufactured by the General Electric Company of England, Ltd.

2. *Human teeth:* Freshly extracted human teeth in good condition were collected from oral surgeons. The teeth were placed in formaldehyde solution shortly after extraction and stored until use. Only bicuspids were used for dentin abrasion measurements, and upper central incisors for enamel abrasion measurements.

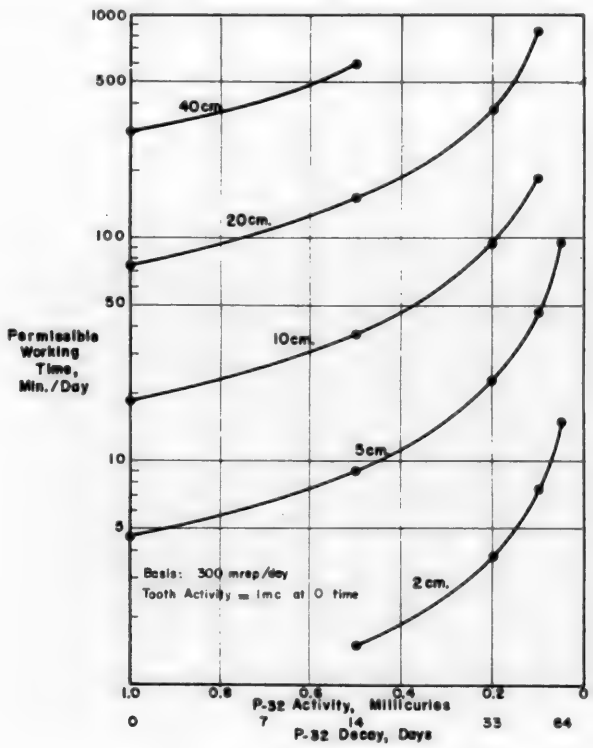


Fig. 3.—Permissible hand exposure.

3. *Suspending liquid:* A 1 per cent aqueous solution of Hercules, medium viscosity, sodium carboxymethylcellulose was used to suspend the abrasive.

4. *Abrasives tested:* The median particle diameter of $CaHPO_4 \cdot 2H_2O$ was 8.5 microns; $CaHPO_4$ (anhydrous), 11.4; $CaCO_3$ (No. 1), 2.0; $CaCO_3$ (No. 2), 1.8; and Insoluble $NaPO_3$, 7.4.

The materials were standard dentifrice grade materials. Median particle diameters were determined by the Andreasen pipette method.[12*]

5. *Sampling:* Pipettes were used for removal of a 2.0 ml. sample of abrasive slurry from the tube after an increment of brush strokes. The pipettes were made from 7 mm. tubing about 8 inches long with one end drawn

*Apparatus available from Fischer Scientific Co., Pittsburgh, Pa. Detailed procedure provided with apparatus.

down to a 3 mm. diameter tip. Each pipette is calibrated with water. Since each sample is weighed, the pipette is used only to ensure narrow limits of variation in the weight of the samples, and thereby to avoid self-absorption variations among the samples, and between the samples and the radioactive standards. A "B.D." Yale 2 ml. syringe is used to draw the sample into the pipette. The sample is discharged into the cupped planchets* made of nickle-plated steel.

6. *Radioactivity measuring equipment:* A Nuclear Instrument and Chemical Corporation Scaler, Model No. 162, was used with an end-window gas flow Geiger-Müller tube operating in the "limited proportion" region at 1,050 volts. A preamplifier was used to eliminate coincidence correction. Precision of counting was of the order of 2 to 5 per cent.

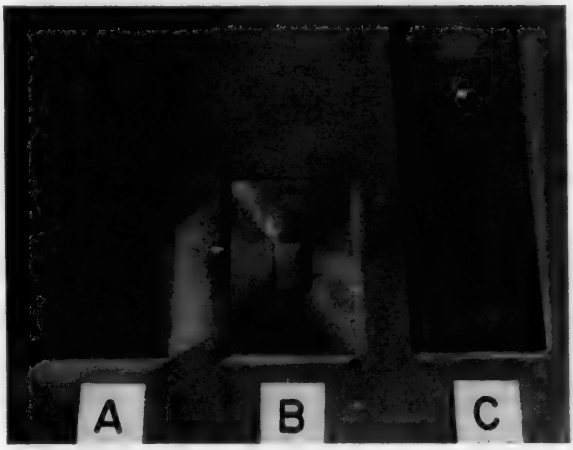


Fig. 4.—Tooth mount mold.

TABLE II

COMPARISON OF ABRASIVES

ABRASIVE	MEDIAN PARTICLE DIA. (MICRONS)	AVERAGE DENTIN ABRASION RATIO*	DENTIN ABRASION RATING†	ENAMEL ABRASION RATING†
$CaCO_3$, No. 1	2.0	1.00	595	22 ± 4
$CaHPO_4 \cdot 2H_2O$	8.5	0.469 ± .030 (± 6.4%)	279 ± 16	2.1 ± .5
$CaHPO_4$	11.4	1.68 ± .114 (± 6.8%)	1000 ± 68	29 ± 4
$CaCO_3$, No. 2	1.8	0.825 ± .052 (± 6.3%)	491 ± 31	–
$NaPO_3$	7.4	1.108 ± .026 (± 2.3%)	659 ± 15	–

*Abrasion Ratio $= \frac{\text{Abrasiveness of unknown abrasive}}{\text{abrasiveness of standard } CaCO_3, \text{ No. 1}}$.

†For convenience, the results were expressed in terms of $CaHPO_4$ having an arbitrary dentin abrasion rating of 1,000, and the others were calculated from the abrasion ratio.

RESULTS AND DISCUSSION

Dentin and enamel abrasion ratings of abrasives (on an arbitrary scale) obtained by using the radioactive tooth test method are shown in Table II.

*Tracerlab, Boston, Mass.

Enamel abrasion is measured by a method similar to that used for dentin abrasion. Because enamel abrades much more slowly than dentin, increments of at least 10,000 strokes between samplings are recommended. Enamel abrasion ratings are computed on the same basis as dentin abrasion, and are of the order of 1 to 5 per cent of the dentin abrasion rating. The enamel abrasion ratings of a few dentifrice abrasives are shown in Table II.

A. *Precision of the Test.*—

1. *Sources of variation:* An analysis of variance was made on the ratios of the dentin abrasiveness of the abrasives to that of the standard $CaCO_3$, No. 1, as listed in Table II. The sources of variance and their magnitudes are shown in Tables III.

TABLE III

RESULTS OF VARIANCE ANALYSIS OF THE ABRASION RATIO CALCULATED FOR A SERIES OF 4 ABRASIVES RUN ON EACH OF 8 TEETH

SOURCE OF VARIATION	VARIANCE
Difference between abrasives	36.18
Difference between teeth	0.16
Difference between runs	0.05
Difference arising from tooth-abrasive interaction	0.27
Difference arising from run-abrasive interaction	0.11
Difference arising from run-tooth interaction	0.04
Residual (unaccounted for by above)	0.13
Total variance	37.58

Ratio: $\frac{\text{Abrasive variance}}{\text{Total variance}} = \frac{36.81}{37.58} = 0.98.$

Hence, 98 per cent of the differences in abrasiveness observed when comparing abrasives by the method described arises from actual differences between the abrasives. The small values of the variance between teeth indicate that the ratio-to-standard method effectively suppressed the differences between the teeth, and the small variance ascribed to tooth-run interaction is evidence that running the standard after every test effectively minimized changes that occurred in the teeth with usage.

2. *Comparison of radioactive-tooth method and depth-of-cut method:* Dentin abrasion results obtained by the depth-of-cut[11] and by the radioactive tooth methods are shown in Table IV.

TABLE IV

DENTIN ABRASION RATING (ON SCALE WHERE $CaHPO_4 = 1{,}000$)

	RADIOACTIVE TOOTH METHOD			DEPTH-OF-CUT METHOD		
ABRASIVE	RATING	σ*	COEF. OF VARIATION†	RATING	σ	COEF. OF VARIATION
$CaHPO_4$	1000	68	6.8	1000	150	15
$CaHPO_4 \cdot 2H_2O$	279	16	6.4	235	80	34
$CaCO_3$, No. 1	595	40	6.8	–	–	–
$CaCO_3$, No. 2	491	31	6.3	635	195	31

*σ = standard deviation.

†Coefficient of variation $= \frac{\sigma}{\text{Mean}} \times 100.$

The coefficient of variation of the radioactive tooth method ranges between 6.3 and 6.8 (for 8 teeth), whereas for the other method the range is from 15 to 34 (8 teeth). The radioactive tooth method required a total of 2,000 brush strokes whereas the depth-of-cut method required 5,000 to 15,000 strokes in order to obtain a measurable cut. If reduced precision is acceptable, 4 different abrasives may be compared with the 8-brush machine in the same radioactive tooth run, running 2 teeth per abrasive. With a pair of results, the average deviation is of the order of 10 to 15 per cent, adequate for most purposes, and the output of the machine is increased by a factor of 4. Most routine testing is carried out using 2 teeth per abrasive.

Experience with the radioactive tooth method and the depth-of-cut method of measuring dentin abrasion has shown that the two methods rank abrasives identically. Although metal strip abrasion tests have not been used in this laboratory, the radioactive tooth method has been applied to materials obtained from manufacturers who use metal strip tests. In agreement with the reports in the literature, it was found that results of the metal abrasion test cannot be universally translated into abrasion of teeth, particularly when a variety of abrasives are to be tested.

The radiochemical tooth abrasion test is much faster than, and shows improved precision over, the depth-of-cut method. Actual running time in testing is reduced by a factor of 5 to 10 by the radiochemical technic, although the time consumed in counting and computation results in an over-all time reduction of about one half to one third. Counting equipment is the only special equipment required and is, at present, readily available, dependable, and relatively inexpensive when time saving in an extensive abrasion testing program is considered. The radioactive species used, P^{32}, is among the less hazardous radioactive materials and, if Atomic Energy Commission recommendations for handling it are followed, no danger is experienced by personnel. Finally, the results obtained are dependable, since they are obtained with human teeth which have undergone little, if any, change in their physical properties. This is an important aspect since the only abrasion tests of comparable convenience and precision utilize metal substrates.

SUMMARY

A procedure for the measurement of either enamel or dentin abrasion by the use of human teeth made radioactive by mild neutron irradiation is described. The method is more precise and faster than former methods dependent upon the measurement of the depth of a groove cut into tooth structure. The results are obtained directly in terms of the amount of human tooth structure worn away by a toothbrush and dentifrice abrasive slurry operating at a known, constant brush pressure and a constant stroke speed. The coefficient of variation of the test is 6-7 when 8 replications are carried out, and 10-15 when only duplicates are run as in the usual routine testing. This is one half to one fourth of the variation involved in the depth-of-cut method and results can be obtained with an expenditure of one half to one third of the time of the former method.

Test results are given for aqueous slurries of dentifrice grade calcium carbonate, dicalcium phosphate (dihydrate and anhydrous), and insoluble sodium metaphosphate.

REFERENCES

1. Miller, W. D.: Experiments and Observations on the Wasting of Tooth Tissue Variously Designated as Erosion, Abrasion, Chemical Abrasion, Denudation, *D. Cosmos XLIX:* 1, 109, 225, 1907.
2. Bunting, R. W., and Rickert, V. G.: Dental Caries, *J. Nat. D. A.* **2**: 258, 1915.
3. Head, J.: Modern Dentistry, Philadelphia, 1917, W. B. Saunders Co., p. 60.
4. Carney, H. J.: Comparative Abrasive Qualities of Five Dentifrices, *J. D. Res.* **3**: cxxxiii, 1921.
5. Wright, H. N., and Fenske, E. L.: Relative Abrasive Properties of the More Commonly Used Dentifrice Abrasives, *J. A. D. A.* **24**: 1889, 1937.
6. Smith, M. L.: The Influence of Particle Size, Shape, Aggregation, and Hardness on the Abrasiveness of Fine Powders, *J. Soc. Chem. Ind.* **54**: 269, 1935.
7. Tainter, M. L.: Abrasiveness of Dentifrices, Proceedings of the Scientific Section of The Toilet Goods Association, Inc., Winter Meeting, 1943-44, p. 24.
8. Epstein, S.: Practical Significance of Abrasives in Dentifrices, *J. A. D. A.* **31**: 400, 1944.
9. Van der Merwe, S. W.: Some Aspects of Modern Dentifrices, *J. D. Res.* **7**: 327, 1927.
10. Tainter, M. L., and Epstein, S.: A Standard Procedure for Determining Abrasion by Dentifrices, *J. Am. Coll. Dentists* **9**: 353, 1942.
11. Manly, R. S.: The Abrasion of Cementum and Dentin by Modern Dentifrices, *J. D. Res.* **20**: 583, 1941.
12. Andreasen, A. H. M.: Berichte des Deutsche Keramischen Gesellschaft **11**: 675, 1930.

ALTERATION OF BLOOD FLOW TO THE TEETH

II. Its Effect on Dental Caries and Relation to Salivary Gland Structure and Function in the Rat

HAROLD E. BREWER* AND JOSEPH C. MUHLER

Indiana University, Bloomington, Ind.

SINCE the vascularity of an organ is important for its proper physiologic maintenance, it seemed significant to study the relationship between decreased vascularity to the teeth and the dental caries experience in the rat. Previous work has suggested that decreased vascularity is associated with an increased caries experience in the rat,[1] and this report is concerned with an attempt to learn more about the mechanism causing this increased caries experience.

EXPERIMENTAL

1. In Series I of this study, a total of 120 weanling rats of the Sprague-Dawley strain were divided equally into 2 groups according to sex and initial body weight. The principal arteries supplying the teeth of animals in the experimental group (*Group 1*) were ligated bilaterally after intraperitoneal anesthesia with pentobarbital sodium. The internal and external maxillary arteries were ligated by methods previously described.[1] The site of ligation of the external maxillary artery was just peripheral to the origin of the submandibular artery. *Group 2* was not operated and served as controls. At the termination of the experimental period of 140 days the animals were sacrificed and the heads were removed for dental caries evaluation by methods previously described.[2] Mandibles of control and experimental animals were fixed in neutral formalin and decalcified in an aqueous solution of Versene,† buffered to pH 6.0, for 20 days. Paraffin sections were cut sagittally and transversely at 8 μ and stained with hematoxylin and eosin.

2. In Series II of this study, 60 male weanling Sprague-Dawley rats were divided equally into 6 groups according to initial body weight. Animals in *Group 1* had the ducts to both right submandibular and sublingual glands ligated, those in *Group 2* had the submandibular-sublingual artery ligated and severed, and those in *Group 3* had both their submandibular and sublingual ducts and artery ligated and severed, respectively. The animals comprising *Group 4* had the right internal and external maxillary arteries ligated and severed and those in *Group 5* had the right submandibular and sublingual vein

This study was supported in part by Grant D-157, National Institutes of Health, United States Public Health Service.

Received for publication April 18, 1958; revised by authors July 3, 1958.

*United States Public Health Service Research Pre-Doctorate Fellow of the National Institute of Dental Research.

†Supplied by Versenes, Inc., Framingham, Mass.

ligated. Animals in *Group 6* served as controls. All of the animals were sacrificed by chloroform at the end of 21 days at which time the submandibular and sublingual glands were removed bilaterally, blotted dry on absorbent paper, weighed together and fixed in Zenker-formol. Paraffin sections were prepared of right and left glands of all animals in each group and stained with hematoxylin and eosin.

3. In SERIES III, 19 adult male and female rats were used in measuring salivary flow. Saliva was collected and flow measurements were made from the submandibular glands because of the relatively large diameter and accessibility of its duct. The duct was cannulated with a 27 gauge, 1½ inch, stainless steel needle. The other end of the needle was inserted into a length of polyethylene tubing. Saliva was collected in a glass tube graduated in 0.007 ml. Pilocarpine nitrate was injected directly into the external jugular vein in order to induce salivary flow. The rate of flow (λ*/10 min.) was determined simultaneously from right and left submandibular glands. The animals in *Group 1* had both the internal and external maxillary arteries ligated. *Group 2* served as controls. By this procedure the effect of ligation of the dental arteries on salivary flow could be compared with flow from unaltered glands. All of the animals in this portion of the study received a stock corn diet (F = 0.5 μg/Gm.) and tap distilled water (F = 0.05 μg/ml.) ad libitum.[3] They were housed in pairs in raised steel cages in an air-conditioned room.

DATA AND DISCUSSION

The dental caries experience in the SERIES I animals is seen in Table I, and indicates a 43 per cent increase in the dental caries experience in the operated animals. The difference is significant at the 0.01 level of confidence. The mean number of molars affected, as well as the severity of the lesions, similarly indicate a higher incidence of dental caries in the operated group. These data corroborate previous work similarly indicating an increase in the dental caries experience in rats with a decrease in blood supply to the molar teeth.[1]

TABLE I
A COMPARISON OF THE DENTAL CARIES EXPERIENCE OF RATS HAVING AN ALTERED BLOOD SUPPLY TO THE TEETH AND UNOPERATED CONTROLS (SERIES I)

GROUP	NO. OF RATS	MEAN NO. OF LESIONS	MEAN NO. OF MOLARS AFFECTED	EXTENT	CARIES INCREASE* (%)
Operated	40	10.7	5.8	2.6	42.8
Control	47	7.5	3.8	1.9	—

*Based upon mean number of lesions.

Although the reason for the increased caries susceptibility in the animals with decreased blood supply is not shown by this study, several factors exist which might be involved. These factors include changes in the physiology of the hard and soft dental tissues. Various investigators have emphasized the

*λ (lambda) = 0.001 ml.

ability of enamel and dentin to permit the centripetal passage of different substances; e.g., organic dyes,[4] inorganic ions,[5] radioisotopes,[6] and tetanus-toxin in presence of glucose.[7] Whether the permeability of the enamel and/or dentin is related to changes in the vascularity of the pulpal organ remains to be investigated. However, this appears to be a logical possibility in view of such findings as those by Wasserman, Blayney, Groetzinger, and deWitt[8] which state that enamel of dogs exposed to radioactive phosphorus showed 10 times as much radioactivity as that of pulpotomized teeth. McCauley and Gilda[9] reported that the uptake of P^{32} in pulpless teeth is only one fourth of the uptake found in normal teeth. Furthermore, the vascularity of the dental pulp may help explain the differences in the effect of certain substances on the permeability of enamel and dentin as determined in the teeth of living animals[7] and in extracted teeth.[5]

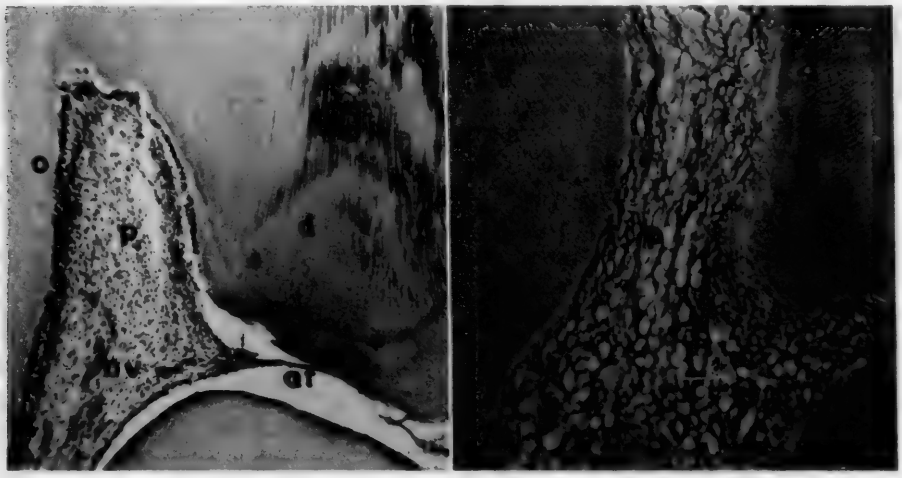


Fig. 1.—Occlusocervical sections through the pulpal horns of mandibular molars of control (*A*) and operated (*B*) rats. Pulp, *p*; primary dentin, *d*; odontoblasts, *o*; artifact, *af*; dentinal caries, *c*; blood vessel, *bv*; secondary dentin, *s*. Magnification (approx.): *A* —×150, *B*—×210.

Fig. 1 shows decalcified sections of the first mandibular molars of a control (*A*) and an animal with decreased vascularity (*B*). Odontoblasts, connective tissue cells, and blood vessels, which are readily apparent in the control pulp, are lacking in the ischemic pulp. What remains of the experimental pulp is a loose network of connective tissue fibers. This type reaction of the pulpal organ to ischemia has been described previously by Butcher and Taylor[10, 11] and was produced in their experiments by retraction of monkey teeth into the alveolar crypt. Alterations in dentinogenesis in the ischemic tooth are indicated by a decreased predentin formation as well as a localized formation of irregular or secondary dentin. These observations tend to confirm the opinion of Shroff[12] that secondary dentin formation results from a decrease in the nutritive supply to the teeth. Carious exposure of the pulp as a cause of the

pulpal changes observed in many of the experimental teeth seems to be precluded by the normal appearance of the primary dentin found in serial sections. Whether the alterations in dentinogenesis is related to the increased dental decay observed in the experimental animals or is merely a reflection of vascular changes in the pulp is not known, but is a problem which requires further investigation.

In addition to direct changes in the dental tissues, the possibility of changes in the salivary glands resulting from the ligation of arteries to the teeth may be a contributory factor. Since the ligation of arteries to the teeth could increase or decrease the amount of blood flow to the salivary glands and, since this alteration in blood flow to the glands, if it occurred, would result in an increase or decrease in gland weight as well as in microscopic changes, these latter criteria were used to evaluate changes in the salivary glands. As it was also possible that, during ligation of the arteries to the teeth, the ducts to the salivary glands or the vein draining them were inadvertently ligated, the effects of impaired venous drainage and loss of salivary flow on salivary gland weight and histology were included in this portion of the study. The submandibular and sublingual glands were chosen as test organs because of their known relationship to dental caries in the rat.

Table II shows the results of ligation of different submandibular and sublingual vessels and ducts on gland weight changes. No difference exists between the mean weights of right and left glands in the same unoperated animal, both weighing 146 mg. However, the data clearly show that ischemia, decreased venous drainage, and loss of salivary flow, resulting from duct ligation, result in varying degrees of salivary gland weight loss. This is indicated both by bilateral comparison of gland weights or by comparison with the controls. The magnitude of weight changes caused by ligation of the vessels and/or ducts to the glands is such that any change in the vascularity of the glands induced by ligation of the internal and external maxillary arteries would be indicated by concurrent weight change. There is no significant difference between the mean weights of right and left glands of animals having the internal and external maxillary arteries ligated dextralaterally.

TABLE II

COMPARISON OF THE EFFECT OF DIFFERENT ARTERIAL LIGATIONS ON THE RATE OF SALIVARY VESSELS AND DUCTS ON SUBMANDIBULAR-MAJOR SUBLINGUAL GLAND WEIGHTS IN RATS (SERIES II)

NO. OF ANIMALS	MEAN WEIGHT GAIN (GM.)	STRUCTURES LIGATED DEXTRALATERALLY	MEAN SUBMANDIBULAR-MAJOR SUBLINGUAL WGT. (MG.) RIGHT	LEFT	P
7	71	None	146 ± 14	146 ± 14	—
8	67	Submandibular, sublingual artery	84 ± 23	187 ± 22	> 0.0001
10	55	Submandibular, sublingual ducts	40 ± 6	153 ± 32	> 0.0001
5	74	Submandibular, sublingual artery and ducts	34 ± 2	206 ± 9	> 0.0001
6	59	Submandibular, sublingual vein	88 ± 37	197 ± 45	> 0.0001
6	59	Internal and external maxillary arteries	141 ± 15	137 ± 14	> 0.70

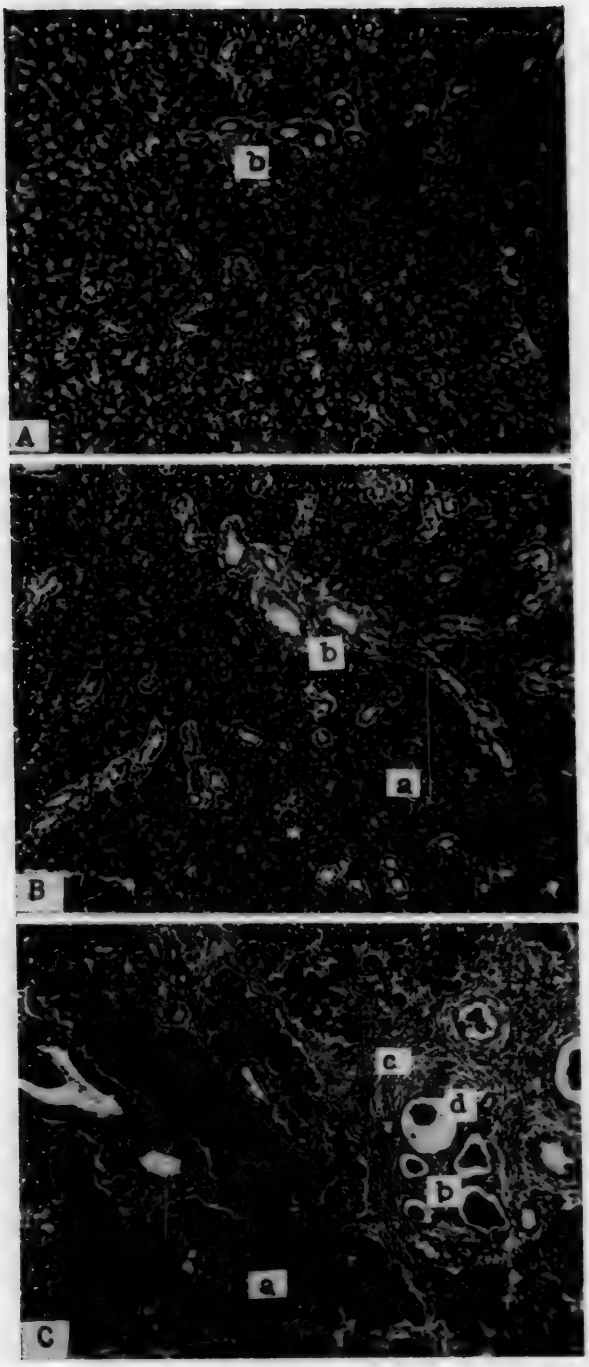


Fig. 2.—Photomicrographs of rat submandibular glands of controls (*A*), animals having the right internal and external maxillary arteries ligated (*B*), right submandibular-sublingual vein ligated (*C*). Approximate magnification—×200. Acinus, *a;* duct, *b;* connective tissue, *c;* degenerated ductal cells and polymorphonuclear leukocytes, *d;* salivary precursors, *e;* blood vessel, *f*.

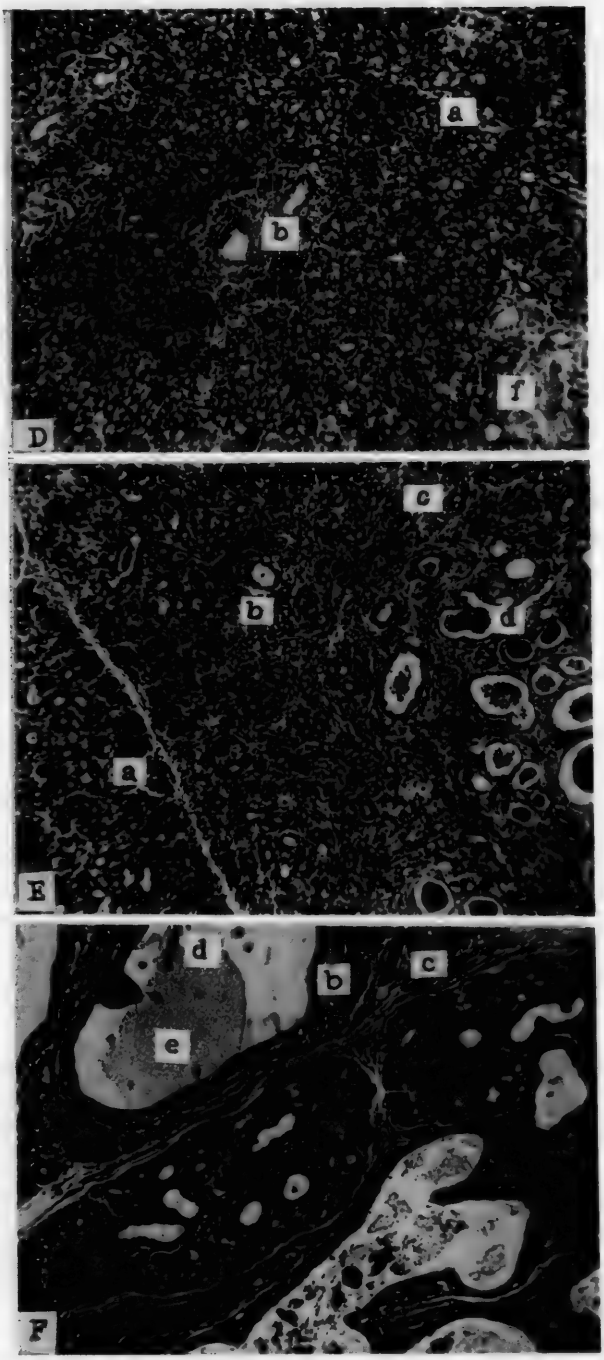


Fig. 2.—Cont'd. Right submandibular-sublingual artery ligated (*D*), right submandibular artery and duct ligated (*E*), and right submandibular duct ligated (*F*). Approximate magnification—×200. Acinus, *a;* duct, *b;* connective tissue, *c;* degenerated ductal cells and polymorphonuclear leukocytes, *d;* salivary precursors, *e;* blood vessel, *f.*

Photomicrographs of sections of glands from each group are shown in Fig. 2. Submandibular and sublingual glands from controls (*A*) and from rats having the internal and external maxillary arteries ligated (*B*) are very similar morphologically. Both acinar and ductal elements are normal. However, the results of the various insults to the vascular supply and drainage are quite different. Section *D* is from a gland which had the artery supplying it ligated. Vascular stasis, connective tissue replacement of gland parenchyma, and degeneration of the duct system are features of the response of the gland to ischemia. Glands which had the excretory duct ligated are represented by section *F*. The fluid-filled, dilated portions of the duct system are the predominant features of this section. Glands having both arteries and ducts ligated, section *E*, exhibit features common to those glands having each operation alone, except for the absence of large dilated ducts. Sections of vein-ligated glands are shown in *C*. The vast increase in connective tissue characterizes the response of the gland to impaired venous drainage.

Table III shows a summary of the data obtained from the collection and measurement of pilocarpine-stimulated saliva from the submandibular glands of adult rats. No statistically significant difference between the rates of salivary flow from right and left glands of the same unoperated animal nor between unoperated glands of different animals is indicated. Although there is a tendency for a higher salivary flow from glands of animals having the arteries to the teeth ligated, no significant difference is shown in the mean salivary flow from right and left glands of the animals in these groups.

TABLE III
COMPARISON OF THE EFFECT OF DIFFERENT ARTERIAL LIGATIONS ON THE RATE OF SALIVARY FLOW IN RATS (SERIES III)

NO. OF ANIMALS	MEAN BODY WGT. (GM.)	ARTERIOLIGATIONS: INTERNAL MAXILLARY RIGHT	INTERNAL MAXILLARY LEFT	EXTERNAL MAXILLARY RIGHT	EXTERNAL MAXILLARY LEFT	SALIVARY FLOW (λ/10 MIN.)* RIGHT	SALIVARY FLOW (λ/10 MIN.)* LEFT	P
10	324	–	–	–	–	168 ± 88	149 ± 86	> 0.60
9	308	–	×	×	–	171 ± 116	135 ± 83	> 0.15

*Pilocarpine-stimulated. Lambda (λ) = 0.001 ml.

SUMMARY AND CONCLUSIONS

In vivo experiments have shown that bilateral interruption of the arterial supply to the molar teeth of weanling rats results in increased dental decay. Additional study has shown that the major salivary glands are unlikely to be related to the increased dental caries experience observed, since no changes in salivary gland function or structure were found to result from ligation of the arteries to the teeth. The necessity of investigating the relationship between the vascularity of the pulp, and enamel and dentin permeability is discussed.

REFERENCES

1. Brewer, H. E., Muhler, J. C., and Fischer, R. B.: Decreased Vascularity of the Dental Pulp. I. The Effects of Unilateral Ligation of Major Vessels to the Teeth and of Combined Ligation—Salivariadenectomy on Dental Caries Experience in the Rat, *J. D. Res.* **36**: 92, 1957.

2. Muhler, J. C., and Shafer, W. G.: Experimental Dental Caries. II. Effect of Desalivation on Dental Caries and Castration and Desalivation on Fluorine Storage in the Rat, *J. D. Res.* **33**: 346, 1954.
3. Muhler, J. C., and Day, H. G.: Effects of Stannous Fluoride, Stannous Chloride and Sodium Fluoride on the Incidence of Dental Lesions in Rats Fed a Caries-Producing Diet, *J. A. D. A.* **41**: 528, 1950.
4. Jansen, M. T., and Visser, J. B.: Permeable Structures in Normal Enamel, *J. D. Res.* **29**: 622, 1950.
5. Brewer, H. E., Muhler, J. C., and Fischer, R. B.: Effects of Fluorides on the Permeability of Human Dental Enamel to Inorganic Ions, *J. D. Res.* **35**: 59, 1956.
6. Wainwright, W. W.: Time Studies of the Penetration of Extracted Human Teeth by Radioactive Nicotinamide, Urea, Thiourea, and Acetamide. I. Diffuse Penetration From the Enamel Surface, *J. D. Res.* **33**: 767, 1954.
7. Berggren, H., and Hedstrom, H.: Experimental Studies In Vivo on the Permeability of Enamel With Particular Regard to the Effect of Sugar Solutions, *J. D. Res.* **30**: 161, 1951.
8. Wasserman, F., Blayney, J. R., Groetzinger, G., and deWitt, J. C.: Studies on the Different Pathways of Exchange of Minerals in Teeth With the Aid of Radioactive Phosphorus, *J. D. Res.* **20**: 389, 1941.
9. McCauley, H. B., and Gilda, J. E.: In Vivo Distribution of Radiophosphorus (P^{32}) in Vital and Pulpless Teeth of a Dog as Indicated by Radioautographs, *J. D. Res.* **22**: 200, 1943.
10. Butcher, E. O., and Taylor, A. C.: The Effects of Denervation and Ischemia Upon the Teeth of the Monkey, *J. D. Res.* **30**: 265, 1951.
11. Butcher, E. O., and Taylor, A. C.: The Vascularity of the Incisor Pulp of the Monkey and Its Alteration by Tooth Retraction, *J. D. Res.* **31**: 239, 1952.
12. Shroff, F. R.: The Physiologic Pathology of Changes in the Dental Pulp. I. Senile Pulp Atrophy, *Oral Surg., Oral Med., & Oral Path.* **6**: 1455, 1953.

DENTAL CARIES IN THE MOLAR TEETH OF RATS

I. Distribution of Lesions Induced by High-Carbohydrate Low-Fat Diets

PAUL H. KEYES

National Institute of Dental Research, National Institutes of Health, Bethesda, Md.

THIS report is based on a survey of the distribution of carious lesions in the molar teeth of several hundred rats maintained on various high-carbohydrate low-fat diets. The study was initiated to resolve some of the differences between previously published descriptions of carious lesions and their prevalence.[1-12]

Stephan[1] reported that certain strains of rats fed rations containing primarily sucrose and skim milk powder developed lesions in occlusal "fissures" and on buccolingual and proximal surfaces. Stephan and Harris[2] concluded, "The similarity of the areas of localization of these carious lesions to the areas of localization of caries commonly found in humans indicates that valid comparisons may be made between them." Losee and Nemes[3] found that Osborne-Mendel rats fed a low-fat, high-carbohydrate ration developed occlusal and surface lesions which approximate a human caries distribution. Mühlemann and co-workers[4] observed all types of lesions and reported the very early (10-day) development of sulcal ("fissure") and proximal lesions in animals fed Stephan's sugar—skim milk powder diet.[2] Dalderup and Jansen,[5, 6] using various purified and nonpurified diets, found that "fissure caries is the most common and is always much more extensive than approximal caries"; cervical and smooth surface attack was observed much less frequently.

By contrast, McClure and co-workers[7-12] reported that rats fed rations containing primarily cornstarch, glucose, and skim milk powder developed lesions located principally along the buccal smooth surfaces: ". . . the majority of the carious areas occurred in the lower teeth and on buccal surfaces, and . . . there were practically no carious areas on the occlusal or lingual surfaces." McClure[7] also reported similar findings in Holtzman rats which were fed a mixture of heat-treated cereals: ". . . caries occurred mostly on buccal surfaces of lower molars. Lingual and mesial surfaces were sometimes involved. Occlusal caries was present . . . but did not appear to originate consistently deep in the sulci of the tooth."

MATERIALS AND METHODS

Over 500 rats were examined in this survey. Of these, approximately 270 animals had received the McClure-Folk Diet 635[9]; about 120, the McClure-Folk Diet 636[9]; and 125 were fed the test diet used for hamsters.[13] Some of the

Received for publication April 19, 1958; revised by author Aug. 1, 1958.

animals fed the McClure-Folk diets[9] had also received various caries inhibitory agents. The results of these experiments with caries-inhibitors have been reported elsewhere.[14] To assess the variability between different stocks of rats fed the same diet under similar conditions, 12 animals from each of 7 different "strains" were given the hamster test diet[13] and distilled water ad libitum for 50 days.

The heads of animals were autoclaved for a few minutes at 15 pounds pressure. Jaws were removed, stripped, and examined both in moist and dry states, and some were stained to enhance distinction of lesions. A few jaws were examined immediately after the animals were killed or after fixation in formalin. All jaws were hemisectioned with a thin steel saw to permit a clear view of sulcal and proximal areas and were examined at 16 to 25 times magnification.

In addition to a careful survey of the appearance and distribution of caries, all lesions were evaluated by two methods. Teeth of Sprague-Dawley rats fed the McClure-Folk rations 635 and 636[9] were scored on the basis of a nonweighted count of areas affected.[15] In Table I the data are summarized for 154 animals with findings reported for sulcal, proximal, and buccolingual areas. The teeth of animals fed the hamster test ration[13] were assessed by a method described in the second paper of this series.[16]

TABLE I

THE TOTAL NUMBER OF CARIOUS AREAS FOUND IN SPRAGUE-DAWLEY RATS FED THE McCLURE-FOLK DIETS 635 AND 636

DIET NUMBER	NUMBER OF ANIMALS	ANIMALS WITH CARIES	AREAS WITH SULCAL (FISSURE) CARIES	AREAS WITH BUCCOLINGUAL SMOOTH LESIONS	AREAS WITH PROXIMAL LESIONS
635	39	39	326	356	106
			(8.4)†	(9.1)	(2.7)
636	37	37	365	210	75
			(9.9)	(5.6)	(2.0)
635+ SLS*	39	38	277	116	67
			(7.1)	(3.0)	(1.7)
636+ SLS	39	39	304	65	7
			(7.8)	(1.7)	(0.2)

*Sodium lauroyl sarcosinate added at 0.5 per cent level.
†Averages are given in parentheses.

Table II presents the data on the average number of enamel units affected for each cavity type in the 7 different stocks of rats examined. These findings are reported on an individual tooth basis in order to better illustrate variations in patterns of attack.

FINDINGS

Jaws examined immediately upon separation from heads revealed rather extensive accumulations of debris and plaquelike material along the gingival crest, often covering considerable portions of the crown. Occlusal sulci were generally filled with soft accumulations also. In most animals, periodontal disturbances were not obvious, but occasionally severe gingival disturbances with debris impaction were apparent.

TABLE II

AVERAGE NUMBER OF ENAMEL UNITS INVOLVED IN THE VARIOUS RAT STRAINS

STRAINS	JAW	SULCAL			BUCCOLINGUAL SMOOTH TYPE			PROXIMAL		MINOR SULCAL COMPLEX*		
		1ST	2ND	3RD	1ST	2ND	3RD	1ST	2ND	1ST	2ND	3RD
Osborne-Mendel	Mand.	9.2	5.8	1.7	1.6	2.2	3.8	0.3	0.3	1.3	1.7	0.9
	Max.	5.6	3.5	1.9	0.6	0.1	0.0	0.0	0.1	--	--	--
Fischer	Mand.	8.3	4.6	4.1	1.2	0.2	3.5	0.0	0.0	1.5	2.4	0.5
	Max.	2.8	2.5	2.8	0.0	0.0	0.1	0.0	0.0	--	--	--
M520	Mand.	7.2	5.8	1.7	1.9	0.8	2.7	0.0	0.0	0.1	1.9	0.4
	Max.	3.6	2.3	1.1	0.0	0.1	0.0	0.1	0.1	--	--	--
Hunt resistant	Mand.	7.4	5.0	1.4	3.1	3.0	1.1	0.0	0.0	0.2	0.8	0.0
	Max.	5.3	2.6	1.7	0.2	0.2	0.0	0.0	0.2	--	--	--
Sprague-Dawley	Mand.	5.7	5.1	1.1	2.6	1.0	3.7	0.2	0.2	0.5	1.1	0.5
	Max.	3.1	2.8	1.2	0.5	0.3	0.1	0.5	0.8	--	--	--
M520 × (A × C)	Mand.	4.1	4.3	1.2	1.4	0.0	0.4	0.2	0.2	0.1	0.6	0.1
	Max.	1.7	1.8	0.7	0.2	0.1	0.0	0.1	0.2	--	--	--
N.I.H. Black	Mand.	2.2	2.5	0.4	0.0	0.0	0.4	0.0	0.0	0.0	0.1	0.3
	Max.	1.0	1.8	0.2	0.0	0.0	0.0	0.0	0.0	--	--	--

*Average number of lesions.

Initiation of caries was observed in the following areas before coalescence took place.

1. *In the major and minor coronal sulci:* Since these lesions seem to be analogous to pit and fissure caries in man, they are usually called "fissure lesions."

2. *On all circumferential surfaces of all molars:* Lesions were observed on all buccal and lingual surfaces, on every proximal surface, and also the anterior and posterior surfaces of first and third molars.

3. *On morsal surfaces of cusps:* Primary involvement of these surfaces was rare. However, occasionally primary lesions were observed; and in certain instances progression from the minor sulcal lesions produced an active morsal component.

4. *Along or below the cervical line:* Carious involvement of the amelocemental junction was found more often in older animals with gingival recession and periodontal disease, but occasionally it occurred in younger animals with severe periodontal disturbances. As this form of caries is not pertinent to this paper, it will not be further discussed.

Major Sulcal Lesions ("Fissure Caries").—Carious lesions which originate at the *bottom* of the major occlusal sulci (Figs. 1 and 2) and in the minor buccal sulci of lower molars (Figs. 2, 3, 4) were the most prevalent type encountered regardless of diet used. This is supported by data in Tables I and II, which show the findings for the various types of lesion and each of the test diets. In the narrow anterior sulci of lower first molars, lesions occasionally originated on sides of the walls in a manner similar to that found by Shaw[17] in cotton rats.* (See Fig. 7*D*.)

*See page 24 in Johansen and Keyes.[18]

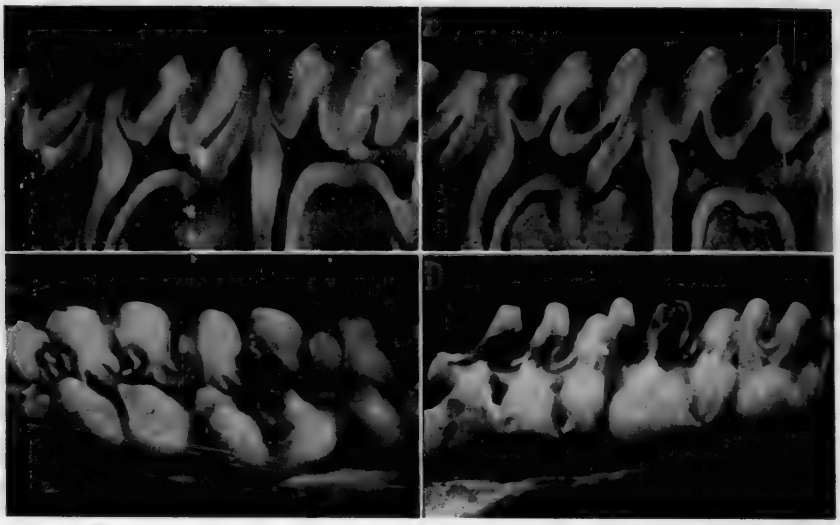


Fig. 1.—*A*, Hemisectioned caries-free lower molars. *B*, Incipient enamel lesions in sulci stained with silver nitrate. *C*, Advanced sulcal lesions with negligible smooth surface caries. These lesions would be scarcely apparent without sectioning. *D*, Active sulcal lesions in maxillary molars with slight caries on buccal surface of the first molar indicated by arrows.

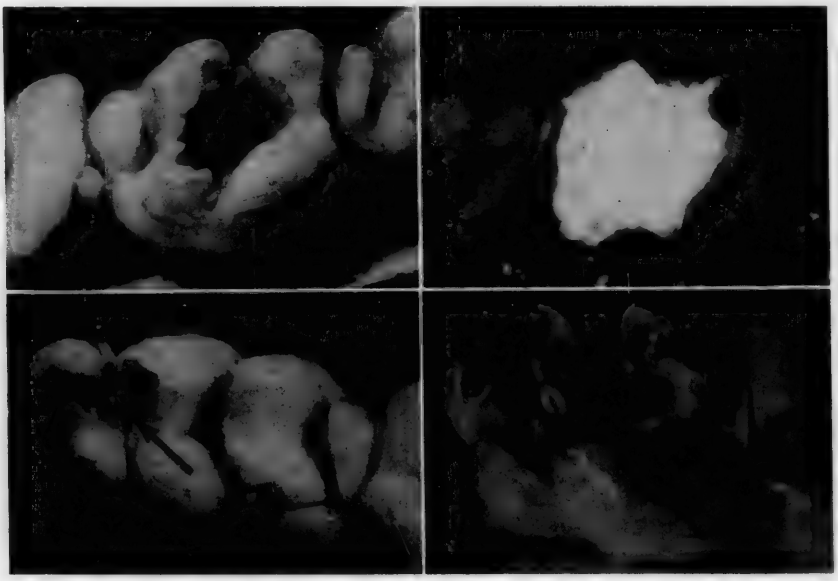


Fig. 2.—*A*, Cavitation in lower second molar which externally appears moderate in extent. Small arrow points to minor sulcal lesion revealed by staining. *B*, Radiograph of tooth shown in *A* after lesion was filled with silver iodide. Little except enamel remains of the crown. *C*, Major and minor sulcal discoloration after staining. Large arrow points to incipient cavitation of morsal surface caused by undermining caries in the sulcus. Small arrows indicate caries in minor sulci. *D*, Advanced lesions of major sulci with caries in minor sulci indicated by arrows.

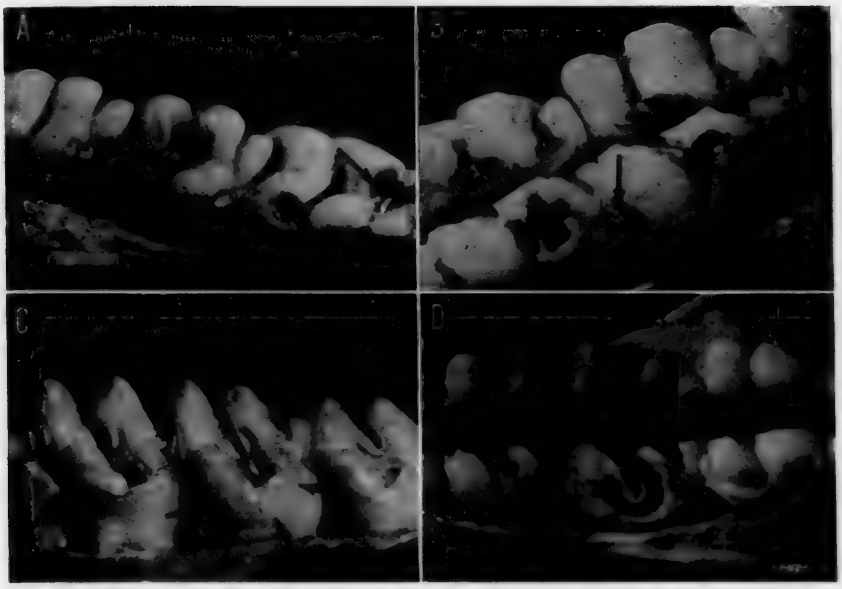


Fig. 3.—*A*, Advanced lesions in minor sulci. The actual extent of the lesions in major sulci cannot be determined without sectioning as illustrated in *B*, *C*, and *D*. Arrow indicates cavitation of morsal surface due to sulcal activity as seen in Fig. 2, *C* and *D*. *B*, Active lesions in minor sulci which have involved morsal surface. Arrows point to additional lesions of similar type. Note evidence of caries activity in all major sulci. *C*, Caries in minor cusps has caused buccal wall to be undermined to extent indicated by arrows. There is no smooth surface caries on this molar. *D*, Buccal wall undermined by caries of major and minor sulci has been removed to show extent of involvement. Note rampant internal caries revealed by sectioning. Small arrow indicates a smooth surface lesion on a third molar.

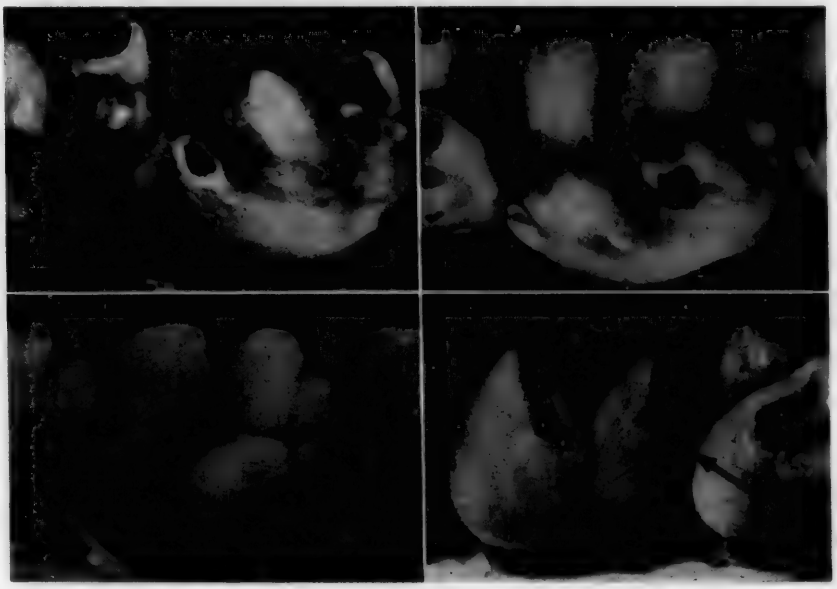


Fig. 4.—*A*, Lesions of minor cusp in lower second molar which has involved the morsal surface. *B*, Lower second molar with lesion in minor sulcus has caused cavitation of the morsal surfaces of both major and minor cusps. *C*, Large arrow indicates coalesced lesions in major and minor sulci. Small arrows indicate additional lesions in minor cusp. *D*, Maxillary second molar with morsal lesion at buccal edge of distal cusp.

It was found that a general examination of occlusal surfaces without grinding or sectioning would not reveal the striking changes that occurred in sulcal areas, nor would painstaking débridement of sulci adequately reveal the full extent of involvement. If the sulcal lesions induced by these fine diets had been diagnosed only on the basis of gross cavitation, they would have had to attain a rather extensive internal magnitude before they became apparent. This is illustrated by Fig. 2 in which the actual extent of the carious lesion is much greater than is apparent from the surface involvement.

The pigmentation associated with caries of enamel in sulci varied from none to intense. When pigmentary changes were minimal, changes in enamel translucency were the most obvious difference between the affected and normal tissue. Occasionally, in sectioned jaws only, minimal changes could be seen in the enamel when specimens were viewed by reflected light at 16 to 25 times magnification, yet the underlying dentin was obviously affected. Sulcal caries was most readily detected in stained jaws which were sliced and dried. The diagnosis of all lesions can be considerably facilitated by immersion of the dried specimens in either benzene or xylene. This clears the specimens instantaneously. Specimens can be placed in a small Petri or "Stender" dish and covered with a clear cover glass during the examination.

Minor Cuspal and Morsal Lesions.—The minor secondary cusps, which are more numerous along the buccal surfaces of lower molars, often became involved in a complex carious lesion. Caries around minor accessory cusps was either localized to the vicinity of the cusp itself, or it was associated with generalized smooth surface changes on the buccal surfaces (Figs. 2, 3, 4, 6*B*). While lesions of this type were induced by all the diets tested, some rations, e.g., the caries-inducing hamster formula,[13] were more conducive to caries in these areas than the others. Also, some strains of rats showed a predisposition for this type of involvement. Variation in cuspal size, contour, and proximity between major and minor cusps probably account for some of these differences in susceptibility.

Incipient carious changes in enamel are often found in the minute grooves (sulci) which separate the minor and major cusps, a finding in agreement with that of Dalderup and Jansen.[5] (Figs. 2*A*, 3, 4.) In many instances, the caries was clearly limited to these minor sulci with slight invasion of the approximating major and minor cusps. At times the gross appearance of lesions suggested a simultaneous, almost primary, involvement of the morsal dentin of these minor cusps, as illustrated in Fig. 4*A*. Here the dentin, upon drying, has separated from its surrounding enamel to leave a small hollow enamel tube. Coalescence of activity in sulcal and morsal areas often caused cavitation which produced considerable undermining of the buccal wall, as illustrated in Figs. 3, *C* and *D*; 4*C*. This form of cavitation either coalesced with generalized buccal caries, or it existed without any evidence of smooth surface involvement. As a typical form of occlusal caries, the pathologic processes appeared to be identical, on a miniature scale, to those which involved the major sulci and cusps, i.e., initiation in the sulcus, invasion of adjacent walls, undermining of the cusps both major and minor, and cavitation of the morsal surface with subsequent progression.

Fig. 5.—*A*, Incipient decalcification of buccal surfaces can be distinguished by spraying moist specimen with air. *B*, Buccal decalcification is identified with white arrows. Lesions in minor sulci are identified with black arrows. *C*, Buccal caries of maxillary first molar. Note decalcification on second molar. *D*, Calculus on maxillary first and second molars with caries lesions in sulci indicated by arrows.

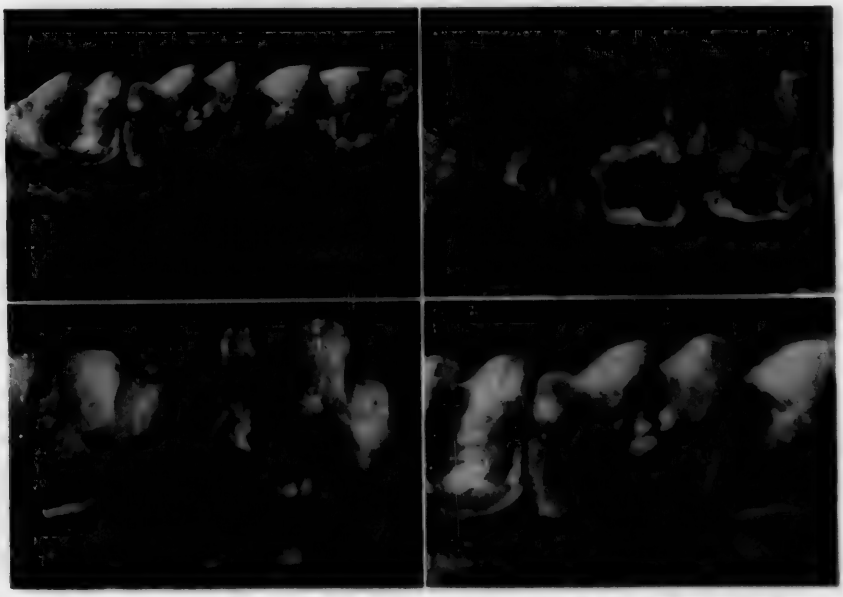


Fig. 6.—*A*, Coalesced caries in mandibular molars. Sulcal lesions have coalesced with proximal, minor cuspal, and smooth surface lesions. Several stages of progression are illustrated in *B*, *C*, and *D*. *B*, Coalescence from activity in minor sulci and smooth surfaces. *C*, Coalesced lesions from caries in major and minor sulci as well as smooth surfaces. *D*, Extensive coalescence of all lesion types.

The *morsal surfaces* of both major and minor cusps are not covered by enamel; nevertheless, under usual experimental conditions initiation of caries on these surfaces has been rare. Fig. 4*D*, however, illustrates an example of a morsal lesion which initiated at the buccal edge of the distal cusp of the maxillary second molar, and specimens are available which illustrate such lesions in all stages of progression. Dalderup and Jansen[5] have observed similar lesions and have come to the conclusion with which we concur, ". . . lesions beginning at these sites cannot be compared with human caries."

Circumferential lesions have been observed on the buccal, lingual, proximal, and anterior surfaces of first molars, and on posterior surfaces of third molars.

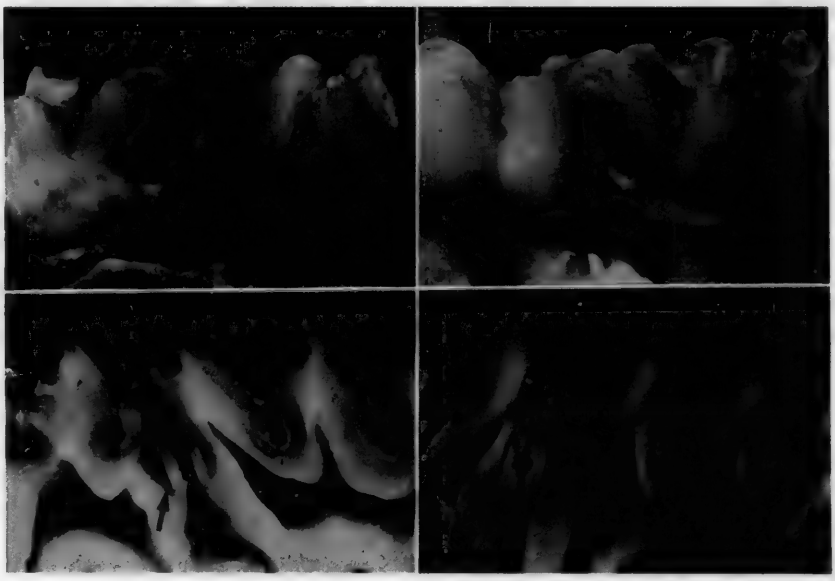


Fig. 7.—*A*, Proximal lesions between maxillary first and second molars. These teeth have been rotated apart to show lesions more clearly. *B*, Gross proximal lesion on distal surface of maxillary first molar. *C*, Proximal lesions in maxillary molars revealed by sectioning. *D*, Proximal lesions in mandibular first and second molars. Small black arrow indicates sulcal lesion which has initiated on adjacent walls of the anterior sulcus of the first molar.

Buccal and lingual smooth surface lesions sometimes originated along the margin of the gingival crest and occasionally in the gingival sulcus. When buccal and lingual surfaces of moist unstained jaws are examined under a stream of compressed air directed through the tip of a 15 gauge needle, enamel lesions become opaque as accelerated drying of the surface blanches areas which have been decalcified (Fig. 5). In the mandible, buccal lesions occurred more frequently than lingual; in the maxilla, the lingual surface tended to be more susceptible.

When the lesions were highly active, considerable penetration of the buccal surface sometimes occurred. In moist specimens the enamel lesions became increasingly opaque and soft. Generally, the underlying dentin was involved

to an extreme degree before cavitation occurred. Dried specimens showed progressive chalkiness and friableness of enamel, and pigmentary changes were more obvious. By the time gross cavitation occurred, sulcal lesions (both major and minor) had generally progressed to the extent that the two coalesced. This resulted in the gross loss of structure seen in Fig. 6.

Proximal lesions were observed on all of the proximal surfaces, especially on the distal of the maxillary first molar and the mesial of the second. Of the several different types of lesions observed in this study the proximal type appears to be the most closely analogous to those seen in man. While gross lesions of the size illustrated in Figs. 7, *A* and *B* could be detected without sectioning, the smaller lesions were more easily detected after staining and slicing (Fig. 7, *C* and *D*). There was a tendency for proximal lesions to occur more frequently in the maxilla than in the mandible.

Jaws from Sprague-Dawley rats fed the McClure-Folk high-carbohydrate low-fat Diets 635 and 636[9] showed a high incidence of proximal and sulcal caries in addition to smooth surface lesions (see Table I). The carious activity in these areas exceeded that on buccolingual surfaces, and it also became apparent that agents tested for caries inhibitory properties were considerably less effective in sulcal and proximal areas than on more open surfaces.

The scores presented in Table II are based on a linear spread of caries in one plane[16] and show the differences that are found when several different stocks of animals are fed the same diet simultaneously. Sulcal activity was particularly high and was not necessarily associated with a high level of smooth surface activity. Depending on the strain of rat, some types of lesions were more active in third molars than in first, and vice versa. The Osborne-Mendel rats developed the most caries and the N.I.H. Black rats developed the least.

DISCUSSION

Stephan and Harris,[2] Losee and Nemes,[3] Mühlemann, Marthaler, König, and Schmid,[4] Dalderup and Jansen,[5, 6] and others have illustrated the complexity of the caries pattern that can be induced in rats, especially by high-carbohydrate, low-fat diets, and they have emphasized the need for presenting a representative assessment of this pathologic process. The results of this investigation agree with and confirm the observations of these investigators. Stephan and Harris[2] have also pointed out that carious lesions in the molar teeth of rats can be placed into several distinct categories and that the relative frequency of each type of lesion will vary with the strain of rat as well as the composition of the diet. Additional support for this conclusion is presented in this study.

The finding of a high incidence of occlusal sulcal lesions in several hundred rats fed the McClure-Folk Diets 635 and 636[9] differs from previous reports[7-12] which have characterized the caries pattern induced by these rations as "primarily smooth surface." However, it should be noted that McClure's reports[7-12] are based on nonsectioned teeth. Our findings give additional support to the observation of Losee and Nemes[3] who concluded that grinding of the crowns is

essential for precise enumeration of occlusal caries. In the present study, after teeth were hemisectioned to permit a diagnosis of sulcal caries with a degree of sensitivity comparable to that applied to buccolingual surfaces, the incidence of sulcal caries induced by Diets 635 and 636[9] exceeded that on smooth surfaces.

McClure's description of occlusal caries[7, 8] has been interpreted as having atypical characteristics[5, 18] as it "did not appear to originate consistently deep in the sulci of the tooth." In the present study all occlusal caries (except morsal lesions in upper second molars) originated deep in sulci of the teeth. However, unlike the occlusal caries induced by coarse corn[18, 19] which may be associated with earlier breakdown of sulcal walls, lesions on these fine diets had undermined the cusps to a considerable extent before gross cavitation occurred, and the first sign of break-through was at the morso-sulcal angle of the cusp. The finding of extensive undermining of cusps prior to gross cavitation confirms other opinions that these lesions are biologically comparable to occlusal caries in man.

The findings on all of the animals examined indicate that a comprehensive survey of caries must include representative lesions of every category, because the prevalence of one type of lesion is not always indicative of the total pattern or the actual extent of caries activity. The wide differences in "susceptibility" among strains suggests that several be examined before broad generalizations are drawn in regard to the effect of diets, various additives, and other experimental variables. For example, in some of the rat strains the conditions on the smooth surfaces differed from those in the sulci to the extent that calculus formed on the circumferential surfaces while caries developed in the sulci (Fig. 5*D*). It is believed that these two processes occurred simultaneously. If a survey of primarily one or two types of lesions be used for purposes of evaluation, an examination of proximal and sulcal areas would give more critical information than one of smooth buccolingual surfaces.

Stephan and Harris[2] have demonstrated the histologic picture of early stages of smooth surface rat caries. Nederveen-Fenenga and Dalderup[20] have described the microscopic details of sulcal caries induced by fine diets. In sulci, penetration was associated with organic lamellae and caused a spreading necrosis in dentin; in the early stages of this process no caries could be observed at the usual magnifications used. Rosebury, Karshan, and Foley[19] have shown a somewhat similar histologic picture in lesions induced by coarse corn diets. All of these findings illustrate that the carious process may penetrate and involve an appreciable amount of underlying dentin without causing gross surface changes.

CONCLUSIONS

1. Almost all strains of rats produced at the National Institutes of Health develop active caries on occlusal and circumferential surfaces of the molar teeth when they are fed high-carbohydrate, low-fat rations. The incidence of lesions and the pattern of caries varied considerably for each type of rat and diet used.

2. Lesions can be placed into the following classifications: sulcal (fissure type), proximal, buccal, lingual, morsal, and cervical or root surface. Sulcal

lesions have been by far the most prevalent. In general, it has not been possible to predict a correlation between the incidence of one type of lesion and another.

3. An accurate appraisal of the caries pattern induced in rats fed the diets used in this study requires grinding or sectioning of the teeth so that sulcal and proximal caries can be diagnosed with a degree of sensitivity equal to that for lesions on buccolingual surfaces.

REFERENCES

1. Stephan, R. M.: The Development of Caries on the Buccal and Lingual Tooth Surfaces of Rats as Well as Proximal and Fissure Caries, *J. D. Res.* **30**: 484, 1951 (Abst.).
2. Stephan, R. M., and Harris, M. R.: Location of Experimental Caries on Different Tooth Surfaces in the Norway Rat. Advances in Experimental Caries Research, Washington, D. C., 1955; Am. A. Adv. Sc., p. 47.
3. Losee, F. L., and Nemes, J. L.: Human Type Caries Distribution in Osborne-Mendel Rats Kept on Heated Skim Milk Powder Diet, *Proc. Soc. Exper. Biol. & Med.* **87**: 429, 1954.
4. Mühlemann, H. R., Marthaler, T. M., König, K. G., and Schmid, H. S.: Tierexperimentelle Caries in 10 Tagen, *Bull. schweiz. Akad. med. Wissensch.* **12**: 528, 1956.
5. Dalderup, L. M., and Jansen, B. C. P.: Evaluation of Dental Caries in the Rat in Non-Decalcified Sections of the Molars, *Internat. Rev. Vit. Res.* **26**: 235, 1955.
6. Dalderup, L. M.: Experimental Dental Caries in Animals, *Ztschr. Prophy. Med.* **3**: 3, 1957.
7. McClure, F. J.: Dental Caries in Rats Fed a Diet Containing Processed Cereal Foods and a Low Content of Refined Sugar, *Science* **116**: 229, 1952.
8. McClure, F. J.: Dietary Factors in Experimental Rat Caries. Advances in Experimental Caries Research, Washington, D. C., 1955, Am. A. Adv. Sc., p. 107.
9. McClure, F. J., and Folk, J. E.: Skim Milk Powders and Experimental Rat Caries, *Proc. Soc. Exper. Biol. & Med.* **83**: 21, 1953.
10. McClure, F. J., and Folk, J. E.: Observations on the Production of Smooth-Surface Rat Caries by Diets Containing Skim Milk and Whey Powders, *J. Nutrition* **55**: 589, 1955.
11. McClure, F. J., and Folk, J. E.: Lysine and Cariogenicity of Two Experimental Rat Diets. *Science* **122**: 557, 1955.
12. McClure, F. J., Folk, J. E., and Rust, J. D.: Smooth Surface Caries in White Rats: Effects of Fluoride, Iodoacetate, Penicillin, Crisco, Butterfat, and a Salt Mixture, *J. A. D. A.* **53**: 1, 1956.
13. Keyes, P. H.: Rampant Dental Caries in Albino Hamsters, I. A. D. R., 35th General Meeting, Atlantic City, N. J., March, 1957, Preprinted Abstracts, p. 68.
14. Zipkin, I., and McClure, F. J.: Effect of Sodium Lauroyl Sarcosinate and Dehydroacetic Acid on Sulcal and Smooth Surface Caries (Buccal-Lingual) in the White Rat, *J. A. D. A.* **55**: 15, 1957.
15. Keyes, P. H.: Recording and Scoring Carious Lesions in Rat Molars, I. A. D. R., 34th General Meeting, St. Louis, Mo., March, 1956, Preprinted Abstracts, p. 18.
16. Keyes, P. H.: Dental Caries in the Molar Teeth of Rats. II. A Method for Diagnosing and Scoring Several Types of Lesions Simultaneously, *J. D. Res.* **37**: 1088, 1958.
17. Shaw, J. H., Schweigert, B. S., Elvehjem, C. A., and Phillips, P. H.: Dental Caries in the Cotton Rat. II. Production and Description of the Carious Lesions, *J. D. Res.* **23**: 417, 1944.
18. Johansen, E., and Keyes, P. H.: Production and Evaluation of Experimental Animal Caries—A review. Advances in Experimental Caries Research, Washington, D. C., 1955, Am. A. Adv. Sc., p. 1.
19. Rosebury, T., Karshan, M., and Foley, G.: Studies in the Rat of Susceptibility to Dental Caries. IV. Further Studies of the Etiology of Fissure Caries, *J. A. D. A.* **21**: 1599, 1934.
20. Nederveen-Fenenga, M., and Dalderup, L. M.: Nutrition and Caries. IV. Histologic Investigation, *J. D. Res.* **35**: 39, 1956.

DENTAL CARIES IN THE MOLAR TEETH OF RATS

II. A Method for Diagnosing and Scoring Several Types of Lesions Simultaneously

PAUL H. KEYES

National Institute of Dental Research, National Institutes of Health, Bethesda, Md.

A NUMBER of different methods are now in use for evaluating experimental dental caries in rats.[1-6] As it is now possible to induce caries on almost all surfaces of the molar dentition of these animals,[6-8] there is need for a system of scoring which will permit the simultaneous evaluation of each of the various types of lesions. In this respect the method of diagnosis and scoring to be described has proved useful. However, it should be mentioned that several of the various methods now employed have features sufficiently similar to permit the adoption of a standardized system of recording which could be more universally applied and understood. A possible approach toward such a method, which could supplement present procedures, will be suggested.

Differences in caries activity have been compared on the basis of the number of lesions in each category without any attempt to score variations in the intensity of attack. For example, the system for recording rat caries used by Stephan and Harris[6] entails a simple count of "gross" lesions revealed by low-power magnification.

Losee and Nemes[7] scored both smooth surface and occlusal sulcal lesions. For the latter type they ground all occlusal surfaces according to the method of Schlack, Howell, and Taylor,[2] to expose the carious lesions otherwise hidden within the coronal grooves. They found that evaluation of occlusal carious areas by grinding resulted in a 20 per cent increase over clinical inspection and concluded that this refinement was essential for precise scoring of occlusal caries.

McClure's procedure[3] does not include either sectioning, grinding, or staining. Lesions along buccal and lingual surfaces are evaluated, but occlusal and proximal forms are included only when gross enough to be "clinically" obvious. Severity scores represent composites which are more qualitative than quantitative.

Dalderup and Jansen[4] describe a detailed technic for evaluating incipient stages of "fissure and approximal caries." Jaws are cut into multiple sagittal sections, stained with fuchsin, and then examined at a magnification of 40 times. Marthaler, Mühlemann, and König[5] allocate lesions into 3 divisions: initial (score A), medium (score B), and severe (score C). They point out that a single caries index is not satisfactory because it will not separate initiation from pro-

Received for publication April 19, 1958; revised by author Aug. 1, 1958.

gression of lesions. They also emphasize the need for sectioning and staining. Additional details of the above method are described by König, Marthaler, and Mühlemann.[9]

Features of the scoring method to be described are:

1. The data recorded indicate the number of animals affected, molars involved, and various types of cavities.

2. The values assigned to quantitate lesion size are reasonably proportional (not weighted), so they can be added, and treated statistically with minimal reservations.

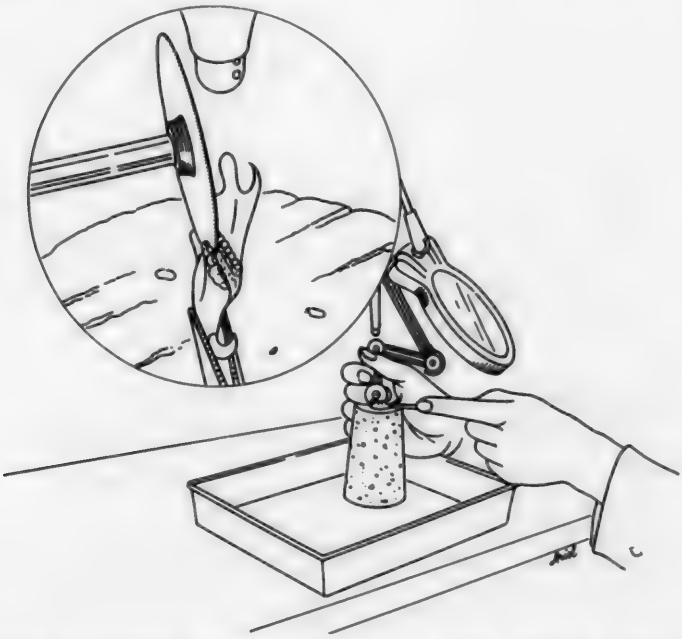


Fig. 1.—Technic of sectioning rat jaws freehand under a small stream of water with a thin steel saw. Inset shows view through magnifying lens.

3. The scores permit immediate comparisons of caries patterns and reduce statistical procedures to the minimum.

4. The values recorded are reproducible when two or more examinations are made by the same observer.

5. The method is easy to use and requires little special equipment.

6. On the basis of the data obtained one can visualize the actual destruction present.

METHODS

Jaws are prepared as follows: The heads are exposed to a dermestid beetle colony which removes the soft tissues within a few days. The skulls are then cleaned in a 1 to 2 per cent solution of ammonium hydroxide for about 30

minutes. They are then put in a saturated solution of Kernechtrot B salt* to which a small amount of 10 per cent neutral formalin has been added to inhibit putrefaction during the staining process. Jaws are stained in this solution for 24 to 48 hours. The stained jaws are rinsed, hemisectioned, and dried. The diagnosis of lesions can be greatly enhanced by placing the dry jaws in a small Petri or "Stender" dish containing either benzene or xylene. This procedure instantaneously clears the specimen and can be recommended. A 2 by 2 inch cover glass of the type used for 35 mm. lantern slides placed over the

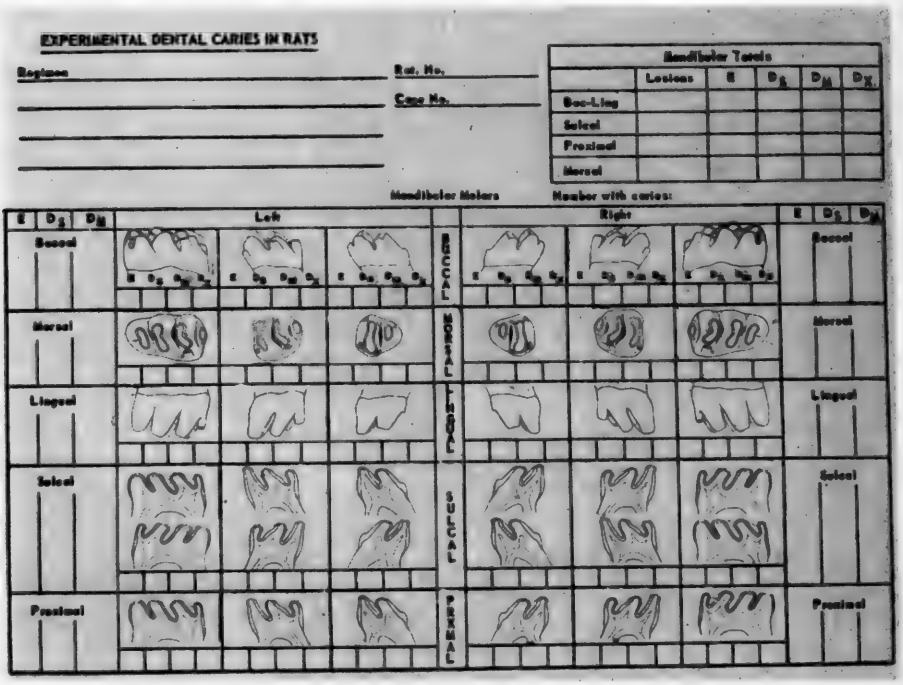


Fig. 2.—One side of the chart designed to provide flexibility in recording. Diagrams of lingual surfaces have been inverted to correspond to teeth as seen after simple rotation of jaw between thumb and index finger. Sulcal diagrams correspond to both halves of sectioned quadrant. Provision has been made for recording the findings in individual teeth, the summation of lesion types, and composite scores.

dishes reduces the presence of vapor from the clearing agent. Smooth surface lesions in unstained jaws have been readily diagnosed by examining moist jaws while they rapidly dried under a stream of compressed air directed from the tip of a 12 or 15 gauge needle. This dehydrates decalcified enamel more rapidly than sound and produces opacities which are readily diagnosed.

Sectioning Technic.—Maxillary and mandibular molars are hemisectioned in a mesiodistal sagittal plane freehand with a steel disc saw, 0.004 inch in thickness and 0.75 inch in diameter, which is mounted on a mandrel held in a

*E. Gurr, Ltd., London, England.

straight handpiece (Fig. 1). The wet jaws, held by a small straight hemostat beneath a slow drip of water, are sliced into equal halves with a standard dental engine running at full speed.

Lesion Types and Evaluation Methods.—

Values are assigned to each type of lesion and are recorded under the appropriate tooth diagram of the record form (Fig. 2). Only for special purposes are lesions outlined on the diagrams as this information has not proved to be of especial value.

The method of assigning values involves judging by eye the linear extent of lesions in one plane and recording the depth of penetration under 4 headings: enamel only (E), slight dentinal (D_s), moderate dentinal (D_m), and extensive dentinal (D_x). Precise measurements are not made of depth as general approximations are more than sufficient for comparisons. The following guides have been used to facilitate estimations of cavity depth. Slight dentinal penetration

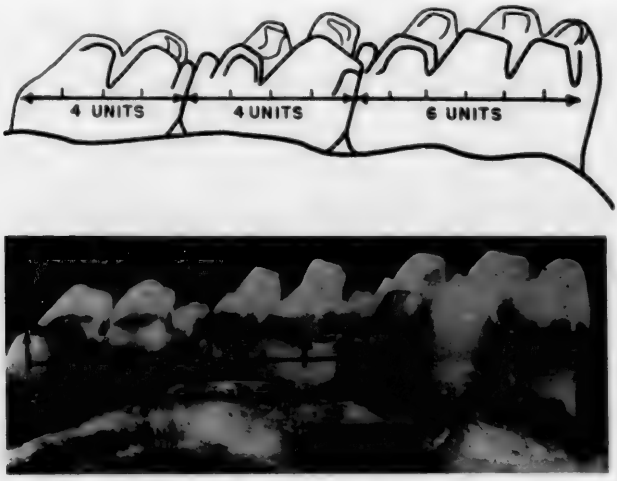


Fig. 3.—Line drawing illustrating the unit scale used for buccal surfaces of mandibular molars. Table I gives additional information for other buccal and lingual surfaces. The buccal smooth surface lesions illustrated in the accompanying figure would be rated as follows: First molar, 3 enamel units (E = 3); second molar, 3 enamel units (E = 3), and one slight dentinal unit (D_s = 1); third molar, 4 enamel units (E = 4), 3 slight dentinal units (D_s = 3), and one moderate dentinal units (D_m = 1). For purposes of illustration dentinal units have been estimated. The usual procedure is to probe lesions before assigning values.

(D_s) has been assigned to all lesions in which the carious process has caused slight separation of the dentin from the overlying enamel up to and including involvement of approximately one-fourth of the dentin between the enamel and pulp chamber. Dentinal penetration between one-fourth and three-fourths of this distance has been called moderate (D_m), while penetration beyond the three-fourth zone has been designated as extensive (D_x).

The buccal involvement of each molar is derived by simply estimating the extent of enamel involved on the basis of the arbitrary scale illustrated in Fig. 3. Mesial surfaces of first molars are omitted unless involvement is limited to this area (a rare condition), in which case this surface is considered the equivalent

of one buccal unit. The distal surface of third molars is considered an extension of the buccal surface and is included in the 4 units assigned. The scale is a sliding one, and two or more small points of attack can be combined into a single figure. Involvement of cervical and root surfaces should not be included in evaluations of coronal caries and, if present, should be placed in a separate category.

If necessary, lesions are probed to permit an estimation of the depth of dentinal involvement. All values are assigned quickly and no half-unit values are used. While there may be slight overlap between two consecutive values, there is negligible overlap between nonconsecutive values. Differences of biologic significance will considerably exceed any overlap involved. Several specific examples of buccal smooth surface involvement are illustrated in Fig. 4, and the scores which would be assigned to these lesions are indicated under the corresponding tooth diagrams. Lingual lesions are similarly treated.

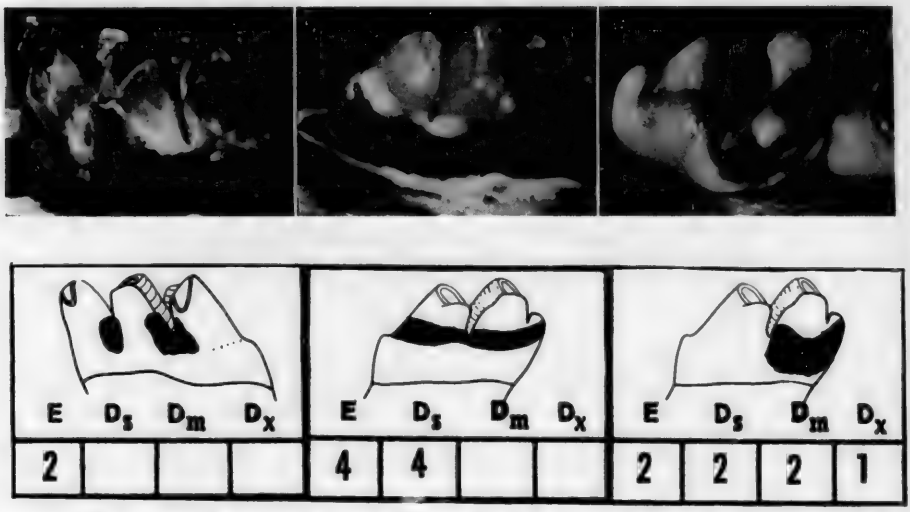


Fig. 4.—Specific examples of buccal lesions and the scores applied. *A*, Maxillary first molar. *B*, Mandibular third molar. If lesion were probed, slight dentinal involvement would be found. *C*, Lesion in mandibular third molar has been excavated with a probe to show that caries has reached pulpal chamber.

When the dentin is extensively involved (D_x), the units of penetration are also recorded in the classification of slight (D_s) and moderate (D_m). Thus, the total number of units in category D_s includes units in D_m and D_x, since units involved in D_m and D_x have necessarily gone through stage D_s. Similarly, units of involvement diagnosed as D_m always are included under D_s. This procedure permits cutting off the values for D_x and D_m if they do not contribute to interpretation of the results. For many studies, counts of enamel involvement alone will provide adequate information for making comparisons. The information on the extent of dentin decayed is, however, readily obtained and enhances the picture of the caries pattern.

The width of lesions is not considered other than as it is related to depth of penetration. If enamel has been undermined, it is scored to the extent of involvement in the underlying dentin so that dentinal scores never exceed those of enamel.

Sulcal Lesions.—The evaluation of caries in the major sulci is achieved quickly by applying a linear estimation to theoretically flattened-out sulci and evaluating depth as previously mentioned (Fig. 5). The number of sulci examined in the mandibular first, second, and third molars is 3, 2, and 1, respectively; and in maxillary first, second, and third molars is 2, 1, and 1, respectively.

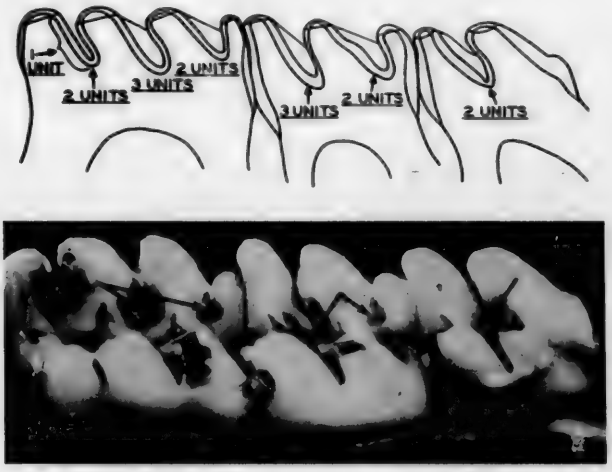


Fig. 5.—Line drawing showing scale of units used for mandibular sulci. See Table I for maxillary values. Sulcal lesions in accompanying figure have been stained and would be rated accordingly: First molar, $E = 4$, $D_s = 4$, $D_m = 1$; second molar, $E = 2$; $D_s = 2$; third molar, $E = 1$, $D_s = 1$. There is smooth buccal caries on third molar only and minor cuspal activity on all molars. Arrows point to sulcal lesions which are combined to yield scores.

An examination of one half of the sectioned jaw is made, and for each tooth a relatively quick approximation is made of the total linear extent of involvement in all sulci taken as a whole, i.e., the several sulci are considered as one. The other sectioned jaw-half is then examined and a final score for each tooth is assigned on the basis of the half having the greater involvement. Table I indicates the total number of linear units assigned to the sulci of each molar.

TABLE I
NUMBER OF LINEAR UNITS ASSIGNED TO EACH MOLAR

	MOLARS					
	MANDIBULAR			MAXILLARY		
LESION TYPES	1ST	2ND	3RD	1ST	2ND	3RD
Buccal	6	4	4	6	4	3
Lingual	6	4	4	6	4	3
Sulcal	7	5	2	5	3	2
Proximal	1	2*	1	1	2*	1

*One mesial and one distal unit.

Fig. 6.—Specific examples of sulcal and proximal lesions which are evaluated in diagrams below. See master chart for orientation. *A*, Sulcal lesions in enamel only. No proximal caries. *B*, Maxillary first and second molars with sulcal and proximal lesions. *C*, Mandibular first and second molars with sulcal and proximal lesions.

The anterior sulcus of lower first molars has been assigned a value of two units and is used as a guide, or standard, for all other sulcal measurements. The lesions illustrated in Fig. 6 have been charted and evaluated on diagrams which correspond to the teeth depicted.

When estimations of carious involvement of sulcal lesions are made, the values assigned to enamel are never less than those given to the underlying dentin.

Proximal Lesions.—Each proximal surface is assigned a linear value of one unit, and depth is recorded under the classifications E, D_s, D_m, and D_x as previously described. The lesions illustrated in Fig. 6 are scored under corresponding diagrams.

Minor Cuspal and Minor Sulcal Lesions.[8]—

These lesions are evaluated on the basis of a simple count of the number observed and also by characterizing the over-all involvement as either slight, moderate, or extensive. Several of these lesions are shown in Fig. 5.

Evaluations of lesions of this type should not be incorporated into *smooth surface scores,* because they are obviously not smooth surface in origin.

Coalesced Lesions.—If experimental periods are overextended, coalescence of lesions may result in massive cavitation. Sulcal, proximal, and smooth surface buccolingual lesions may cause extensive coronal destruction. Most workers agree that a more accurate and meaningful evaluation can be made when experimental periods are terminated before extensive coalescence occurs. If extensive cavitation involves large portions of the crown, a satisfactory classification would be slight, moderate, severe, and total coronal destruction.

TABLE II
COMPARISONS OF WET AND DRY ESTIMATIONS OF BUCCAL ENAMEL CARIES

GROUPS	RIGHT			LEFT		
	Wet	Dry	P	Wet	Dry	P
I	7.68	7.80	>0.9	7.16	7.12	>0.9
II	2.80	2.77	>0.9	2.80	2.73	>0.9
III	6.47	6.13	>0.7	5.83	5.87	>0.9
IV	4.03	4.03		4.10	4.10	

Reproducibility of Measurements.—In order to check reproducibility of measurements, two assays were made of smooth surface and sulcal lesions in several groups of rats. In wet jaws smooth surface changes were first scored along mandibular buccal surfaces under the small air spray. Under the heading of enamel involvement (E) the mesiodistal spread of cavitation and enamel opacity was estimated, and the number of units affected for each tooth was recorded directly on the tape of an adding machine. It was possible to evaluate the extent of enamel caries on buccolingual smooth surfaces of approximately 150 animals in about 8 hours. The same jaws were then stained and re-examined in more detail, and findings were recorded on the charts illustrated. The second procedure was considerably more time consuming, because all lesions were probed

to determine the extent of dentinal penetration. However, as indicated in Table II, data obtained by the two methods are statistically indistinguishable for carious involvement of enamel. For many comparisons an assessment of enamel caries would be adequate. For other comparisons the more detailed examination is helpful since it gives information on dentinal penetration.

Sulcal caries was first scored in wet-stained jaws immediately after hemisectioning and then rescored after the teeth had thoroughly dried. The values obtained from the wet and dry examination of sulcal lesions are presented in Table III. While the similarity in values obtained by each type of examination indicates that the two methods would yield comparable results, lesions in the air-dried specimens are more easily detected, as indicated by the fact that in 22 of 24 comparisons, values for air-dried specimens slightly exceeded those for wet jaws. All air-dried values which are higher than wet values have been underlined.

TABLE III
REPRODUCIBILITY OF ESTIMATIONS OF ENAMEL INVOLVEMENT IN SULCI

	LOCATION	RIGHT				LEFT			
	MOLARS	FIRST		SECOND		FIRST		SECOND	
GROUP	ESTIMATION	1st	2nd	1st	2nd	1st	2nd	1st	2nd
I	Mandibular	3.25	3.52	2.29	2.52	3.28	3.74	2.32	2.70
	Maxillary	1.56	1.91	1.08	1.25	1.68	2.24	1.12	1.32
II	Mandibular	3.56	3.53	2.19	2.27	3.38	3.64	2.07	2.14
	Maxillary	1.53	2.25	1.07	1.26	1.87	2.18	1.07	1.32
III	Mandibular	.55	1.18	.17	.36	.45	1.04	.14	.25
	Maxillary	0	.21	0	.03	.10	.34	0	0

DISCUSSION

The data derived by the simple methods described have been adequate for comparisons of differences in caries activity. Differences of biologic importance will far exceed the small variations between similar types of lesions, or those slight inaccuracies inherent in the assignment of scores. When differences between the samples are obviously large, it seems unnecessary to use multiple tests for significance. When statistical analyses are made, however, caution is necessary in assigning levels of probability as this type of data may be skewed. The nature of the study will determine whether scores for each of the lesion types should be compiled on an entire jaw basis or on an individual molar basis. In some studies, data on individual molars permit comparisons of importance.

The present method purposely avoids the use of those composite caries scores which include several different lesion types. The comment of Cox, Dodds, Dixon, and Matuschak[10] is pertinent to the general principle that scores derived from different types of lesions should not be pooled into a composite, because processes involved may not be comparable: "We have hesitated to use total count, as it involves the summation of numbers that are non-linear; e.g. a cavity of size 2 is not twice the size of one with a score of 1."

Some form of sectioning, or grinding, is *essential* to permit an appraisal of sulcal and proximal areas that is as sensitive as that applied to buccal and lingual surfaces. A method based on the estimation of one type of carious lesion, e.g., smooth surface caries, may not be reliable for comparisons, especially if coalescence has occurred in one of the groups. With coalescence, sulcal and

EXPERIMENTAL DENTAL CARIES IN RATS

			RIGHT				LEFT				TOTAL		
			E	SD	MD		E	SD	MD		E	SD	MD
SMOOTH BUCCAL LINGUAL	MAND	1											
		2											
		3											
	MAX	1											
		2											
		3											
MINOR SULCAL	MAND	1											
		2											
		3											
MAJOR SULCAL	MAND	1											
		2											
		3											
	MAX	1											
		2											
		3											
PROXIMAL	MAND	1											
		2											
		3											
	MAX	1											
		2											
		3											

TOTALS
MAND - MAX

E	SD	MD

TOTALS

E	SD	MD

TOTALS
MAND - MAX

E	SD	MD

TOTALS
MAND - MAX

E	SD	MD

Fig. 7.—Example of the type of chart which might be used to report findings obtained by most of the various scoring systems now in use.

other lesions are obvious and included in scores; whereas, without coalescence, very active sulcal and proximal lesions are less obvious, and remain undetected and unscored. Thus, in the first instance, scores represent two or more entities; and in the other instance, only one. Such scores do not give a true picture of the disease process involved and may exaggerate differences.

Severity scores derived on a qualitative basis do not represent similar entities and are not quantitative. Means, standard deviations, and percentage differences cannot be derived in a strictly mathematical sense.

The fact that several of the present-day rat-scoring technics have features of basic similarity suggests that a more standardized method might be adopted to supplement procedures now in use. A recording method could be devised which would entail little change, if any, in most of the present scoring systems and data could be presented in one supplementary table.

As a possible approach to a standardized method, information recorded on the master chart is transferred to the form illustrated in Fig. 7. Smooth surface caries is largely assessed on the basis of buccal surface activity with lingual surfaces added, if sufficiently affected to require special representation. Proximal caries is assessed on the basis of all the maxillary and mandibular locations; sulcal caries on the basis of all the major sulci in the maxillary and mandibular molars (24 in all). Minor sulcal lesions are noted as previously mentioned. Involvement in each of the locations is characterized as (1) none; (2)enamel only (E); (3) slight dentinal (SD); and (4) moderate dentinal (MD). Space has been provided for noting extensive dentinal involvement, but it is rarely used and is not specifically labeled on the form. The tabulation of data from these forms gives information which everyone can immediately understand. If investigators could agree on the scale to be used, i.e., what degree of involvement is to be included under the respective headings, it is believed that the final compilation would more closely depict the extent of caries in experimental groups than any now used.

SUMMARY

1. The various types of carious lesions found in the molar dentition of the rat can be evaluated by estimating the linear spread in one plane and by characterizing the depth of penetration.

2. In order to determine the extent of proximal and sulcal lesions some form of sectioning or grinding is essential.

3. These features have been incorporated into a standardized system of scoring which is consistent with presently used methods and which offers the advantage of describing and distinguishing differences in the distribution and extent of carious lesions. It is hoped that this system may find universal adoption.

REFERENCES

1. Johansen, E., and Keyes, P. H.: Production and Evaluation of Experimental Animal Caries. A Review, Advances in Experimental Caries, Philadelphia, Pa., 1955, Am. A. Adv. Sc.

2. Schlack, C. A., Howell, S. R., and Taylor, B. L.: A Procedure for Preparing and Examining Rat's Teeth for Dental Caries, Naval Med. Res. Inst., Bethesda, Md., Proj. Rep. No. 5, 1947 (Project No. X-418, now NM 008 002).
3. McClure, F. J., and Folk, J. E.: Skim Milk Powder and Experimental Rat Caries, *Proc. Soc. Exper. Biol. & Med.* **83**: 21, 1953.
4. Dalderup, L. M., and Jansen, B. C. P.: Evaluation of Dental Caries in the Rat in Nondecalcified Sections of the Molars, *Internat. Rev. Vit. Res.* **26**: 235, 1955.
5. Marthaler, T. M., Mühlemann, H. R., and König, K. G.: Zur Planung und Auswertung tierexperimenteller Kariesstudien, *Schweiz. Monatsschr. f. Zahn.* **67**: 755, 1957.
6. Stephan, R. M., and Harris, M. R.: Location of Experimental Caries on Different Tooth Surfaces in the Norway Rat, Advances in Experimental Caries Research, Washington, D. C., 1955, Am. A. Adv. Sc., p. 47.
7. Losee, F. L., and Nemes, J. L.: Human Type Caries Distribution in Osborne-Mendel Rats Kept on Heated Skim Milk Powder Diet, *Proc. Soc. Exper. Biol. & Med.* **87**: 429, 1954.
8. Keyes, P. H.: Carious Lesions in Rat Molars. I. Distribution of Lesions Induced by High-Carbohydrate Low-Fat Diets, *J. D. Res.* **37**: 1077, 1958.
9. König, K. G., Marthaler, T. M., and Mühlemann, H. R.: Methodik der kurzfristig erzeugten Rattenkaries, *Deutsche Zahn-, Mund-, und Kieferheilkunde* **29**: 99-127, 1958.
10. Cox, G. J., Dodds, M. L., Dixon, S. F., and Matuschak, M. C.: Experimental Dental Caries. III. A System of Recording Corn Meal Caries in Rat, *J. D. Res.* **18**: 469, 1939.

NORMAL DEVELOPMENT OF TOOTH BUDS CULTURED IN VITRO

WILLIAM LEFKOWITZ AND PATRICIA SWAYNE

College of Dentistry, The Ohio State University, Columbus, Ohio

CELL culture founded on the ability of cells to survive and proliferate in vitro, has made major contributions to the field of experimental biology. Organ culture, concerned with histo-differentiation and function of cells, has similarly enhanced the field of embryology. Rudimentary organs have been successfully cultured in vitro. Among these is the fetal tooth bud.[1-3, 5, 7-10, 12, 14]

Tooth bud culture presents multitudinous problems. It is a mixed organ composed of ectodermal and mesodermal derivatives which have different nutritional requirements.[8] In vivo tooth formation presents a harmony of development between the tooth bud components[4] which must be maintained if organ culture is to be successful. Other associated problems relate to aberrations in the gross morphology of the explant,[5, 8] decrease in size,[1, 2, 8] and the common occurrence of degeneration in tooth bud culture. This report offers a partial solution to the existing problems and establishes an easy, consistent, culture technic which may be utilized as a limited experimental method.

PART I

MATERIALS AND METHODS

All tooth bud explants, dissected from 19-day rat fetuses,[6] were derived from the Sherman albino strain. Experiments were designed to test planting technics and nutritional requirements of the explant. The medium consisted of rat serum and/or embryo extract in a plasma coagulum with Tyrode's solution. The combined medium contained 0.005 phenol red. Thrombin was added to facilitate clotting. Cultures were examined at 4-day intervals, at which time photographs were taken and the pH adjusted to 7.6. Explants were examined histologically at intervals after 12 days of cultivation. The maximum time of cultivation was 36 days. The total number of explants cultured was 348. The following experiments were performed.

1. Two hundred and twenty-six explants were cultivated in a plasma coagulum containing rat-embryo extract. A supernatant nutritional fluid contained the same concentration of rat-embryo extract in Tyrode's solution. The concentration of embryo extract ranged from 6 to 25 per cent. A parallel study

Presented at the 35th General Meeting of the International Association for Dental Research, Atlantic City, N. J., March 23, 1957.

This study was supported in part by a grant [D168(C3)] from the National Institutes of Health, Bethesda, Md.

Received for publication April 28, 1958.

was made of 40 explants substituting 10 per cent rat serum for the embryo extract. All explants were bathed in situ with Tyrode's solution after 4 days of cultivation. Fresh supernate was then added. The explants were not transplanted.

2. Forty-four explants were cultured according to the above methods but were not retained in the original clot. At 4-day intervals the explants were dissected from the coagulum and outgrowth cells, washed in Tyrode's solution and transplanted in similar fresh medium.

3. Eighteen tooth buds were cultured in a fluid medium in tubes containing 20 per cent rat serum and 1 per cent rat-embryo extract in Tyrode's solution. The explants were redissected from the outgrowth and transplanted to similar fresh medium at 4-day intervals.

4. Twenty explants were cultured in an 80 per cent plasma and 20 per cent Tyrode's solution coagulum without a supernate. These were redissected from the plasma and outgrowth washed in Tyrode's solution, and transplanted in similar fresh medium at 4-day intervals.

OBSERVATIONS

Outgrowth Cells.—Regardless of the technic of cultivation or the nutritional medium employed, all tooth bud explants exhibited both epithelial and fibroblastic cell outgrowth which arose from the peripheral cells of the explant, survived, and proliferated as cell cultures. The outgrowth cells were predisposed to grow on surfaces. In the Carrel-flask technic they proliferated mainly on the surface of the clot, the clot-glass interface, or on the interface of a double clot (Fig. 1). Epithelial outgrowth was short lived. It took the form of sheets (Fig. 1) or budding laminae (Fig. 2), depending on its origin. Ultimately, the lamina type outgrowth formed microcysts.[13] Both types degenerated in 6 to 8 days. Fibroblasts survived for a longer period of time and occupied the area originally pervaded by the epithelial outgrowth. After approximately 4 to 8 days of cultivation, some of the fibroblasts transformed into monocyte-like wandering cells (Fig. 3) at which time lysis of the coagulum occurred (see Fig. 7). The influence of degenerated outgrowth cells and lysis of the clot was presumed to be undesirable.

Position of Explant in the Clot.—Examination of untransferred tooth buds planted in the clot revealed that they did not maintain their original position. In approximately 5 days they settled to the clot-glass interface, frequently degenerating before the termination of the experiment (Fig. 4).

In an effort to overcome this problem, the tooth buds were planted on the surface of a thicker clot, permitted to adhere, and then covered with a second clot. Despite the thickness of the clot, the explant continued to settle to the surface of the glass. Survival improved although the explant flattened (Fig. 5). The outgrowth cells as well as the outer enamel epithelium assumed the covering characteristic of cell culture.

Immediately upon planting, the ellipsoidal tooth bud was opaque when examined with transmitted light. Permitted to remain in the original coagulum, it soon became translucent, permitting identification and observation

of the tooth bud components.[5] Upon examination of the specimen and sectioning, it was learned that these explants had contacted the surface of the flask and reduced in thickness to a few layers of cells. While development continued, the morphology of the surviving explants had been altered (Fig. 5). The surface cells, in this abnormal environment, modified to primitive forms and ultimately degenerated. Accordingly, it was possible to establish the positional relation of the explant to the glass surface by the optical characteristics of the explant in situ.

Shrinkage.—Tooth buds cultured in vito reduce in size.[1, 2, 8] This seems paradoxical since mitosis was seen to continue in the area of the cervical loop. Accordingly, the reduction in size must be construed as due to shrinkage. Diminution in size was noted in both degenerated and surviving explants. There was no relation between reduction in size of the explant and degeneration.

Explants were measured photographically. The method is crude since only the perimeter was used for comparison. Explants which became flat in contact with the glass surface often appeared to increase in size. Dimensional change was also related to the surface of the spheroidal explant exposed to the camera. The observations on shrinkage were, in the main, subjective.

When the tooth germ was cultured in a medium containing 5 per cent or more rat-embryo extract in the supernate, the reduction was maximal.[8] Replacing the embryo extract with 10 per cent rat serum resulted in lesser shrinkage (Fig. 7). Using 20 per cent rat serum and 1 per cent rat-embryo extract produced results similar to 10 per cent rat serum.

Supernate and Fluid Media.—The cultivation of tooth buds in a coagulum with a fluid nutritional supernate resulted in degeneration of more than 50 per cent of the explants regardless of the nutritional medium employed. The need for a plasma coagulum, already established,[15] had been supplemented by experimentation which established the desirability of organ culture in a nutritionally reinforced coagulum.[7]

To test further the effect of fluids on organ culture, tooth buds were cultivated in a fluid medium. Eighteen tooth buds were transplanted into tubes containing 20 per cent rat serum and 1 per cent rat-embryo extract in Tyrode's solution. The tubes were shaken periodically to prevent the explants from adhering to the glass. The method failed. In this study all explants degenerated.

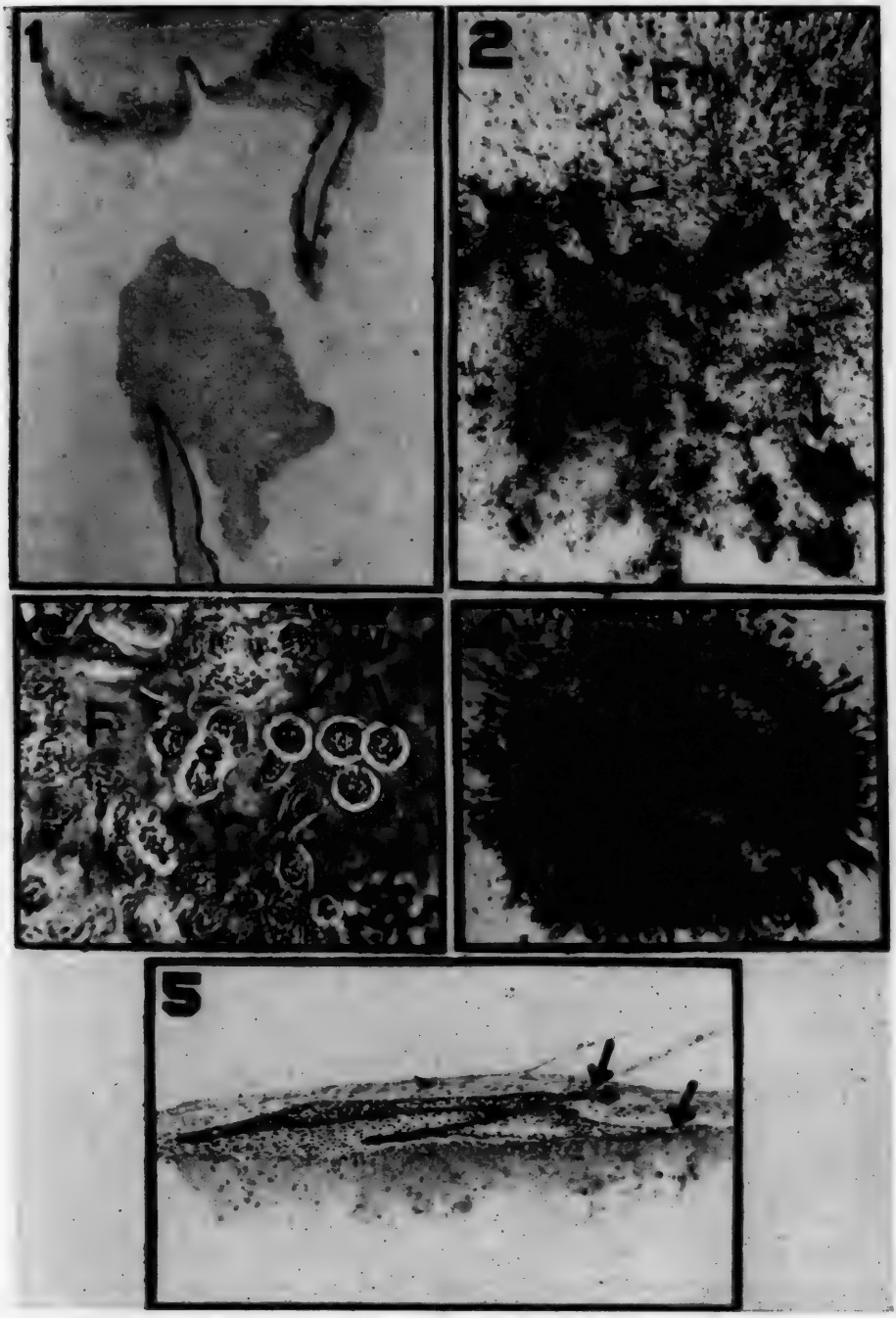
Fig. 1.—Section of epithelial outgrowth covering surface of coagulum. (Orig. mag. ×435.)

Fig. 2.—Photograph of living epithelial outgrowth. Arrows point to budding epithelial proliferation. Fibroblasts (*F*). (Orig. mag. ×50.)

Fig. 3.—Photograph of living monocyte-like wandering cells (*W*) and fibroblasts (*F*). (Orig. mag. ×720.)

Fig. 4.—Tooth bud cultured for 16 days on surface of a 0.67 c.c. coagulum covered with a second coagulum. Explant settled to glass surface. Tooth bud degenerated. Outgrowth survived as cell culture. (Orig. mag. ×45.)

Fig. 5.—Section of an explant cultured for 16 days on surface of a 1.5 c.c. coagulum covered with a second coagulum. Arrows point to outer enamel epithelium migrating at clot-glass interface. (Orig. mag. ×90.)



Figs. 1-5.—*For legends, see opposite page.*

After dissection, tooth buds were generally immersed in Tyrode's solution for varying periods of time. In addition, they were again washed, either in the clot or after dissection during transplantation. There was a correlation between the length of time explants were washed and degeneration. Later experiments reduced the time of washing to one-half hour after dissection and transplantation.

Nutritional Media.—A further test of the need for serum or embryo extract was made by cultivating the tooth buds in plasma and Tyrode's solution. Neither embryo extract nor serum was added. Twenty dissected tooth buds cultivated in this medium degenerated between 4 and 7 days.

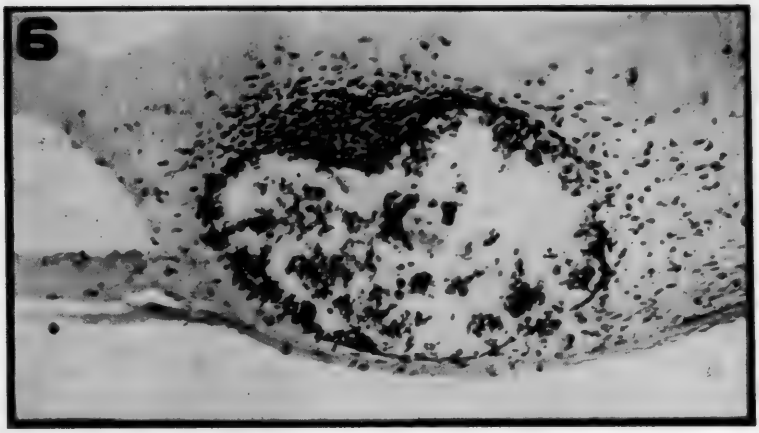


Fig. 6.—Tooth bud cultivated in medium containing 12 per cent rat-embryo extract for 16 days. Not transferred. Note presence of wandering cells in papilla and areas of degenerated fibroblasts. Survival of enamel epithelium good. (Orig. mag. ×65.)

Nutritional media were limited to homologous embryo extract and/or serum added to chicken plasma and Tyrode's solution. Early efforts utilized 6 per cent rat-embryo extract in the medium.[5] In order to test the effect of rat-embryo extract, concentrations at 12 per cent and 25 per cent were used in the coagulum and supernate. With the increased concentration of embryo extract, there was a more abundant cell culture of peripheral explant cells. Wandering cell transformation, followed by simultaneous lysis of the clot and degeneration, occurred in 4 to 8 days. Cultivation beyond this period resulted in a sudden pH reduction below 7.0. Histologic examination revealed complete or partial degeneration of the cells of the papilla. Contrary to observations of the mixed outgrowth cells, survival of the enamel epithelium surpassed that of the papilla (Fig. 6).

A parallel experiment using 10 per cent adult rat serum instead of embryo extract established serum as a more desirable component of the medium. In those cases where a supernate was used, the degeneration was the same as in the embryo extract studies. The outgrowth cells, mainly fibroblasts, were quantitatively reduced and survived for approximately 10 days after which

monocyte-like wandering cells appeared and lysis of the clot occurred. Of those explants which survived, the development surpassed that found after cultivation in medium containing embryo extract (Fig. 8). There was remarkable harmony of development of the enamel organ and dental papilla. Under higher magnification (Fig. 9), elongated ameloblasts which were separated from the odontoblasts by a basement membrane were observed. A small area of degenerated fibroblasts was present in the dental papilla. This study suggested the nutritional merit of rat serum.

pH Change.—All flasks were adjusted to pH of 7.6 after planting. There was little, if any, pH change in these studies except in those cases where complete degeneration of the explant occurred. The general observation was that the pH dropped to 7.2 in a 4-day period for surviving explants. At this time the pH was readjusted to 7.6. Of particular interest was the higher concentration of rat-embryo extract studies in which there was a sudden drop of pH below 7.0 after 10 days of cultivation in some of the explants. Degeneration of the fibroblasts occurred at this time.

Part II

Materials and Methods

Previous experiences stimulated the following alterations in technic and media. Eighty-seven explants derived from 19-day rat fetuses were cultivated in a coagulum containing a biologic nutritional medium, chicken plasma and phenol red in Tyrode's solution. Bovine thrombin in Tyrode's solution was added to facilitate clotting. Two different nutritional media were used.

Medium A consisted of:

10% rat serum
10% Tyrode's solution containing 0.05 phenol red
10% Tyrode's solution containing 10 units of bovine thrombin
70% fresh frozen heparinized chicken plasma

Medium B consisted of:

20% rat serum
1% rat embryo extract
10% Tyrode's solution containing 0.05 phenol red
10% Tyrode's solution containing 10 units of bovine thrombin
59% fresh frozen heparinized chicken plasma

Dissected explants were washed in Tyrode's solution for one-half hour prior to planting. One and one-half cubic centimeters of either formula were pipetted into a Carrel flask and permitted to clot. The tooth buds were planted on the surface of the coagulum and placed in the incubator for about 2 hours. At that time the explants had adhered to the coagulum and 1 c.c. of the same medium was pipetted over the surface and permitted to coagulate. The pH was adjusted to 7.6. A sterile cotton plug was placed in the neck of the flask to absorb any liquefaction of the coagulum to avoid the equivalent of a supernate. The flask was stoppered, sealed with paraffin, and incubated at 37° C. The explants were photographed 24 hours later.

Fig. 7.—Photographs of living tooth bud cultured in medium containing 10 per cent rat serum. *A*, One day after planting; *B*, 4 days; *C*, 8 days; *D*, 12 days; *E*, 16 days; *F*, 19 days, demonstrating increase in size from 5 to 16 days. Nineteen days (*F*) after cultivation compares favorably with one day (*A*). Note lysis of coagulum after 4 days of cultivation. (Orig. mag. ×36.)

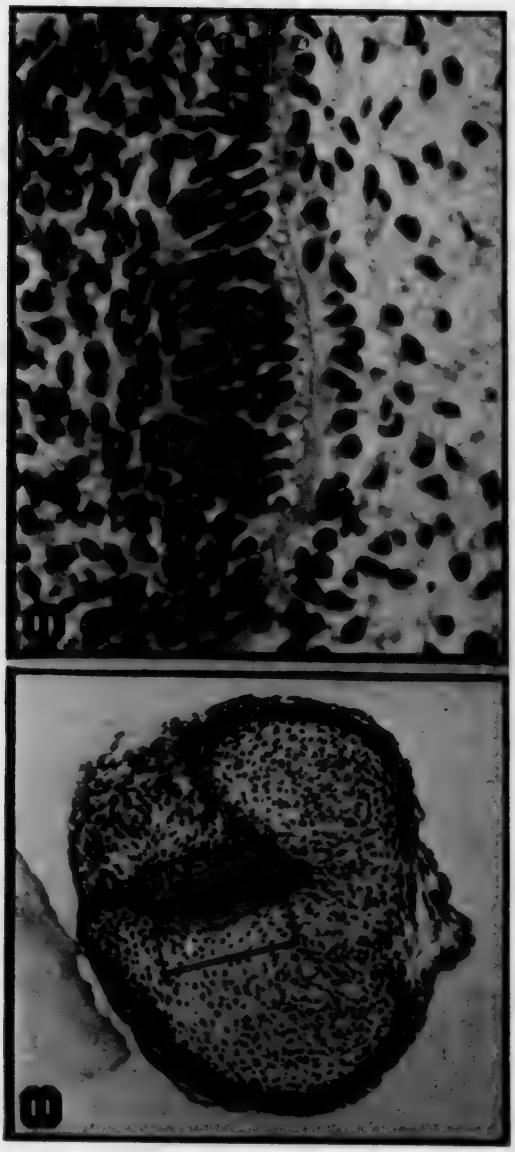


Fig. 8.—Section of tooth bud cultivated for 20 days in medium containing 10 per cent rat serum. Explant not transplanted. Note survival and normal development. (Orig. mag. ×190.)

Fig. 9.—Higher magnification of boxed area in Fig. 8. Note elongated ameloblasts, thickened basement membrane, and odontoblasts. In vitro development equivalent to 3 days in vivo. (Orig. mag. ×840.)

Fig. 10.—Tooth bud cultivated in medium containing 10 per cent rat serum (formula A), according to method described in Part II. Photographs of tooth bud in the flask at the following intervals: *A*, one day; *B*, 4 days; *C*, 8 days; *D*, 12 days; *E*, 15 days; *F*, 19 days; *G*, 22 days; *H*, 26 days. (Orig. mag. ×45.)

At approximately 4-day intervals the tooth germ was photographed and the pH recorded. The coagulum was removed from the flask and the explant dissected from the coagulum and outgrowth, washed in Tyrode's solution containing either 10 per cent rat serum for those in formula A or 20 per cent rat serum and 1 per cent rat-embryo extract for those in formula B. The period of washing did not exceed one-half hour. The tooth buds were then transplanted into fresh medium of the original formula. The pH was adjusted and the explants photographed and incubated.

OBSERVATIONS

Outgrowth Cells.—The transplantation of explants made it impossible to compare the outgrowth of this procedure with untransplanted tooth buds beyond the first 4 days. A minimal amount of outgrowth appeared within 24 hours after planting, consisting of fibroblasts and epithelial cells. At 4 days the rapidly proliferating epithelium outgrew the fibroblasts (Figs. 10, *B* and 11, *B*). Explants in formula B containing rat-embryo extract exhibited more epithelial outgrowth than did those in formula A. It occurred mainly in the area of the future occlusal surface. In areas where the stratum intermedium was at the surface of the dissection, the outgrowth took the form of budding epithelium (Fig. 11, *C*). The epithelial outgrowth recurred after the first dissection. Small lamina-like projections of epithelium continued for about 16 days (Fig. 11, *E*). After the first transplantation very little epithelial outgrowth occurred with formula A (Fig. 10). There was no degeneration of epithelial outgrowth between transplantations, although microcysts appeared in the budding epithelial outgrowth at this time.

Fibroblastic outgrowth was minimal during the first two transplantation periods. Transplants in formula A showed much fibroblastic outgrowth at the end of 4 days, after which it was minimal. At about 20 days there was an increase in the fibroblastic outgrowth (Fig. 10, *G* and *H*). Formula B produced less fibroblastic outgrowth than formula A. The amount of fibroblastic outgrowth declined after 16 days of cultivation. Few monocytes were observed at the end of each 4-day culture period and lysis of the coagulum did not occur. The major part of the outgrowth when present was at the interface of the two coagulums.

Position of Explant in the Coagulum.—Tooth buds planted in a double coagulum and transplanted at 4-day intervals did not settle to the clot-glass interface. They maintained their spheroidal form throughout the period of cultivation as observed during transplantation. The explants upon gross examination retained their original opacity unlike that found after flattening (Figs. 10 and 11).

Shrinkage.—Shrinkage of the explant was reduced above that occurring in all other methods and media. The initial shrinkage occurred up to about the second transplantation or 8 days of cultivation. Following this period there was a progressive increase in the size of the explant which at no time achieved the photographic dimensions of the original explant. At 20 days of cultivation there was a slight decrease in size (Figs. 10 and 11).

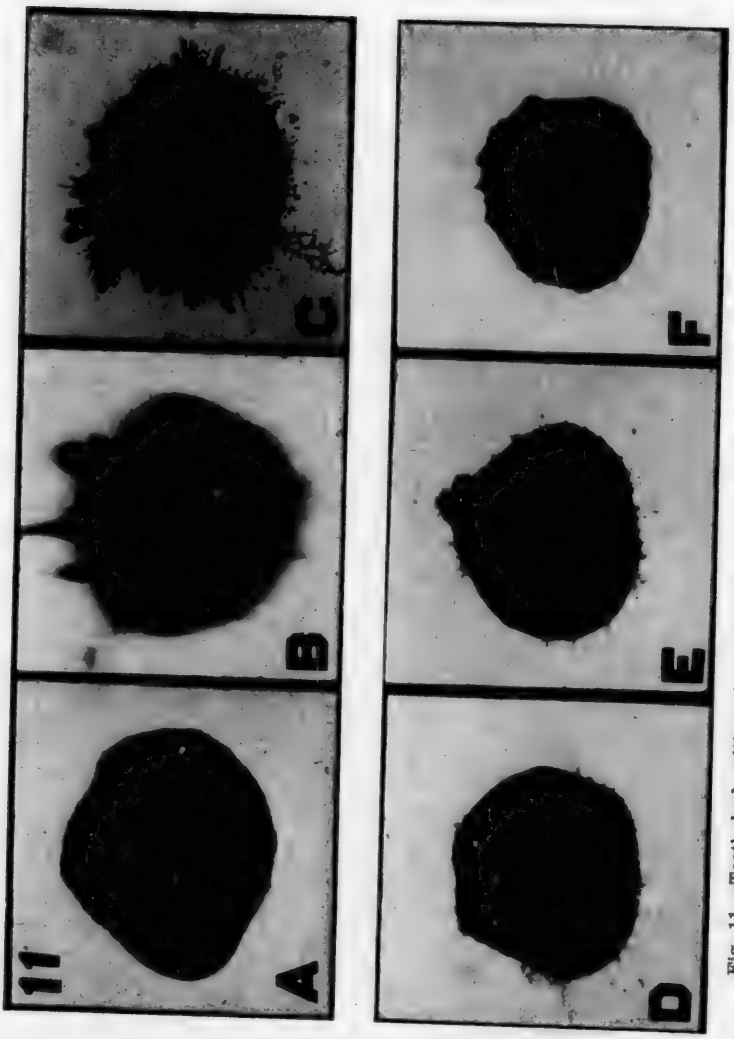


FIG. 11.—Tooth bud cultivated in medium containing 20 per cent rat serum and 1 per cent rat-embryo extract (formula B) according to method described in Part II. Photographs of tooth bud in the flask at the following intervals: *A*, one day; *B*, 4 days; *C*, 8 days; *D*, 12 days; *E*, 16 days; *F*, 20 days. (Orig. mag. ×45.)

pH Change.—There was a consistent reduction in pH from the time of planting to transplanting. The original medium was adjusted to pH 7.6. After 4 days of cultivation, the pH had dropped to below 7.0. After transplantation, the fresh medium was adjusted to pH 7.6. The pH change was consistent in its gradual reduction for both formulas, A and B. No degeneration of the explant cells occurred.

Histologic Examination.—Explants were taken for histologic examination at 4-day intervals beginning on the twelfth day of cultivation. The average period of cultivation was 20 days. Upon removal of the explants from the clot, the normal ellipsoidal morphology of the explant was noted. All cellular elements of the tooth bud were viable at the termination of the experiment as determined by histologic examination. The development of both ectodermic and mesodermic derivatives was harmonious.

Fig. 12 is a low-power photomicrograph of a tooth bud which had been cultivated for 15 days in formula A. By contrast with the developmental stage of a 19-day rat-embryo tooth bud,[4] the equivalent of 4 days in vivo development had occurred. The morphologic anatomy was normal. Under higher magnification (Fig. 13), the cellular elements and matrix formation may be seen. The elongated ameloblasts presented Tomes' processes and a minute layer of pre-enamel matrix. The odontoblasts were fully differentiated and a narrow zone of predentin had formed. Cultivation for 20 days in the same nutritional medium (Fig. 14) showed further development of the tooth bud. The morphology of the components and cellular survival was maintained. Under higher magnification (Fig. 15), it was observed that the development was approximately equivalent to 5 days in vivo.

By contrast, development with formula B demonstrated a slight advance over that with formula A. Fig. 16 is a low-power photomicrograph of a section of a tooth bud cultivated for 16 days in formula B. The section, cut obliquely, does not demonstrate the dentinoenamel outline which is one of the standards for determining development. All cellular elements were viable and the explant showed normal development. Under higher examination (Fig. 17), the differentiation of the peripheral cells of the stratum intermedium into a cuboidal form had already begun. There was a change in polarity of the ameloblasts with formation of Tomes' processes. Differentiation of odontoblasts and the formation of the basement membrane were also evident, demonstrating the equivalent of 5 days in vivo development. Cultivation for 20 days (Fig. 18) demonstrated no further advance in development and formation. Higher magnification (Fig. 19) showed normal ameloblasts, Tomes' processes, and a narrow increment of the pre-enamel matrix. Fully differentiated odontoblasts and an increment of predentin were present. The development was equivalent to 5 days in vivo development. Cultivation for 16 days in formula B provided the same development as that occurring with 20 days of cultivation in formula A.

DISCUSSION

While the described technic of organ culture establishes a limited period of normal development in vitro, the problems associated with it are not completely resolved. Of major significance is the lack of calcification of the

formed enamel and dentin matrix. The limited development may express a relationship between matrix formation and calcification. The amount of matrix formed is quantitatively equivalent to the amount of pre-enamel matrix and predentin which occurs before these tissues are calcified in vivo. Failure to form additional increments may be dependent upon lack of calcification of the already formed layers. Efforts to alter the media to induce calcification have, to date, been unsuccessful. The hypothesis that calcification of previously formed increments is essential to further formation is under investigation.

The existing concept[11] that transplantation of organs cultured in vitro is undesirable must be re-examined in the light of the evidence presented. Short-lived outgrowth cells which undergo necrosis are known to release intracellular enzymes which hydrolyze the cells. Lysis of the coagulum, which occurs at the same time, may be caused by the same enzyme or others released at the time of necrosis. The effect of such enzymes on the organ cells is not known but supports the concept that outgrowth cells should be removed before necrosis occurs. As experienced in this study, the transplantation of the organ at intervals was beneficial.

As previously reported,[7] explants in a plasma coagulum exhibited the equivalent of 2 days in vivo development after 8 days of in vitro cultivation. The present study verifies a previous report that there is sufficient residual energy in the explant to provide a relatively minor degree of development. The addition of rat serum and rat-embryo extract to the plasma coagulum is essential for continued development. It may now be postulated that plasma plays an insignificant role as a nutritional medium.

The described method of measuring growth is inadequate for organ culture. Shrinkage of the explant was reduced by the technic described in Part II, although prolonged cultivation continued to show a progressive reduction in size. It is possible that frequent dissection of the outgrowth may be responsible for the reduction in size since there was evidence of growth, particularly at the dentinoenamel junction. Another possible cause may be

---

Fig. 12.—Section of a tooth bud cultivated 15 days in medium containing 10 per cent rat serum. Transplanted. Angle of section does not illustrate development of the dentinoenamel outline. Harmonious development of components. In vitro development equivalent to 4 days in vivo. (Orig. mag. ×66.)

Fig. 13.—Higher magnification of boxed area in Fig. 12. Note Tomes' processes, preenamel matrix and increment of predentin. (Orig. mag. ×480.)

Fig. 14.—Section of a tooth bud cultivated 20 days in medium containing 10 per cent rat serum. Transplanted. Harmonious development of components and dentinoenamel outline. In vitro development equivalent to 5 days in vivo. (Orig. mag. ×66.)

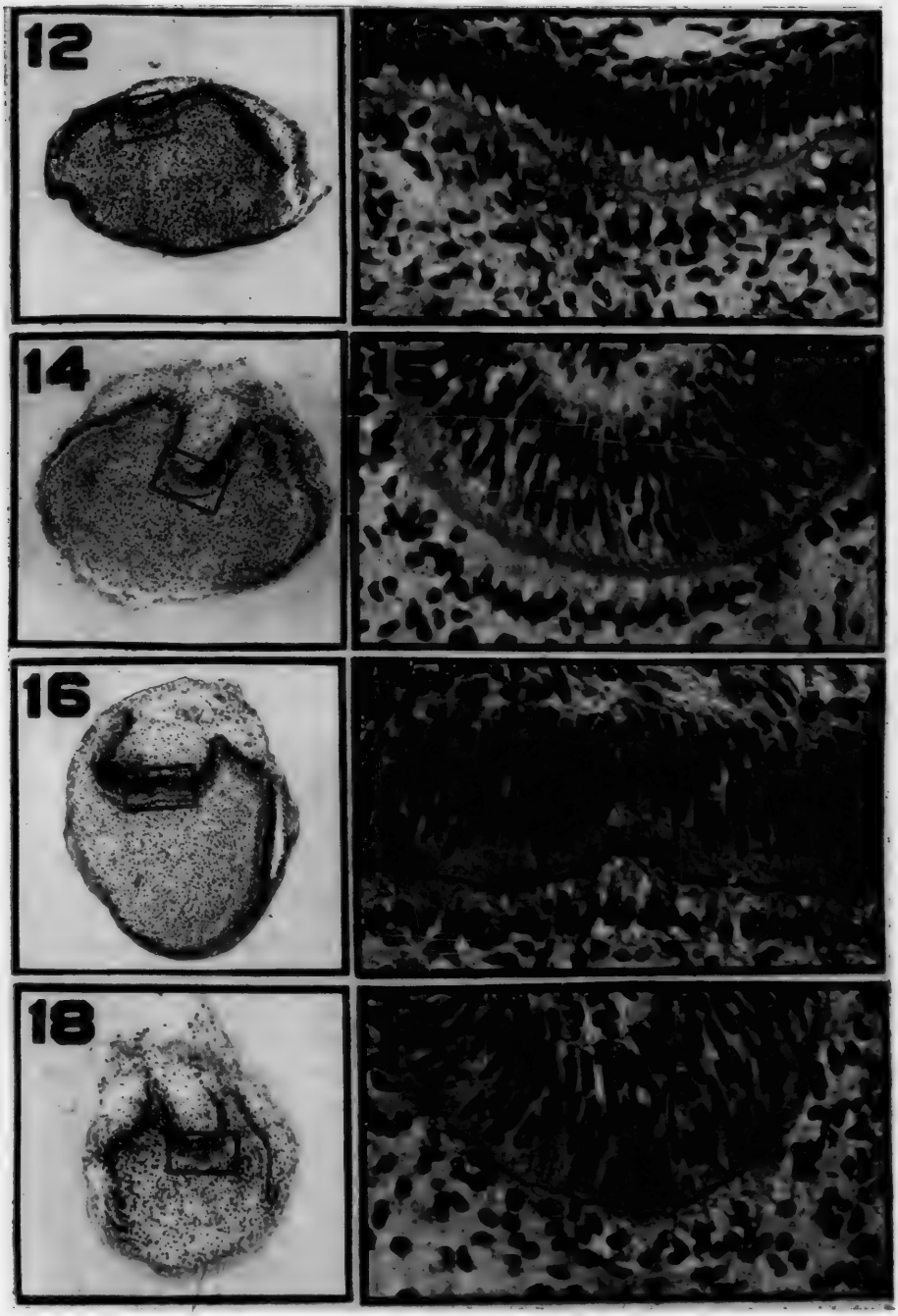
Fig. 15.—Higher magnification of boxed area in Fig. 14. Note Tomes' processes, preenamel matrix and increment of predentin. (Orig. mag. ×480.)

Fig. 16.—Section of a tooth bud cultivated 16 days in medium containing 20 per cent rat serum and 1 per cent rat-embryo extract. Transplanted. Section cut obliquely. Harmonious development of components. In vitro development equivalent to 5 days in vivo. (Orig. mag. ×66.)

Fig. 17.—Higher magnification of boxed area in Fig. 16. Note Tomes' processes, preenamel matrix, and increment of predentin. Development equivalent to that seen in Fig. 15. (Orig. mag. ×480.)

Fig. 18.—Section of a tooth bud cultivated 20 days in medium containing 20 per cent rat serum and 1 per cent rat-embryo extract. Transplanted. Harmonious development of components. In vitro development equivalent to 5 days in vivo. (Orig. mag. ×66.)

Fig. 19.—Higher magnification of boxed area in Fig. 18. Development same as that seen in Fig. 17. (Orig. mag. ×480.)



Figs. 12-19.—*For legends, see opposite page.*

the failure to maintain isotonicity of the nutritional medium. Hypertonic media appeared to be associated with diminution in size of the explant. This area of investigation is being pursued.

Despite existing problems, the method described provides an acceptable experimental technic for testing many hypotheses. Problems in calcification and the effect of nutrients on the development of first molar tooth buds of a 19-day rat embryo to its developmental equivalent of 2 days after birth should provide essential information. In addition, the technic lends itself to storage of tooth buds for a minimum of 20 days. The small amount of development which occurs during this period of cultivation suggests a useful method for storage prior to transplantation.

CONCLUSION

A simple and effective method for in vitro cultivation of tooth buds has been presented which fulfills in part the requirements for an experimental method.

REFERENCES

1. Glasstone, S.: The Development of Tooth Germs "In Vitro," *J. Anat.* **70**: 260, 1936.
2. Glasstone, S.: A Comparative Study of the Development "In Vivo" and "In Vitro" of Rat and Rabbit Molars, *Proc. Roy. Soc., London*, Sect. Biol. **126**: 315, 1938.
3. Glasstone, S.: The Development of Halved Tooth Germs. A Study of Experimental Embryology, *J. Anat.* **86**: 12, 1952.
4. Lefkowitz, W., Bodecker, C. F., and Mardfin, D. F.: Odontogenesis of the Rat Molar, *J. D. Res.* **32**: 749, 1953.
5. Lefkowitz, W., Mardfin, D. F., and Bodecker, C. F.: Cultivation of Rat Molar Tooth Germs in Carrel Flasks. I, *J. D. Res.* **33**: 189, 1954.
6. Lefkowitz, W., and Mardfin, D. F.: Dissection of Rat Molar Tooth Germs, *J. D. Res.* **33**: 201, 1954.
7. Lefkowitz, W., and Swayne, P.: The Cultivation of Tooth Germs in an Embryo Extract-Free Medium, *J. D. Res.* **35**: 440, 1956.
8. Lefkowitz, W., and Swayne, P.: In Vitro Cultivation of Rat Molar Tooth Germs. II, *J. D. Res.* **35**: 523, 1956.
9. Losee, F. L.: Method of Growing Rat Tooth Germs "In Vitro," Using the Depression Slides, *U.S. Nav. M. Bull.* **41**: 758, 1943.
10. Nuckolls, J.: Lobular Development and Calcification in the Tooth, *J. California D. A.* **17**: 73, 1941.
11. Parker, R. C.: The Cultivation of Tissues for Prolonged Periods in Single Flasks, *J. Exper. Med.* **64**: 121, 1936.
12. Pinkerton, H., and Boyle, P. E.: Cultivation "In Vitro" of Tissues Removed From the Enamel Organ and Dental Pulp, *J. D. Res.* **15**: 221, 1935.
13. Robinson, H. B. G., and Lefkowitz, W.: The Ameloblastomatous Potentiality of Odontogenic Epithelium Demonstrated in Tissue Culture, *Oral Surg., Oral Med., & Oral Path.* **11**: 630, 1958.
14. Szabō, G.: Studies on the Cultivation of Teeth "In Vitro," *J. Anat.* **88**: 32, 1954.

A HISTOCHEMICAL STUDY OF THE ATTACHED GINGIVA IN PREGNANCY

SAMUEL TURESKY, BEVERLY FISHER, AND IRVING GLICKMAN

Tufts University, School of Dental Medicine, Boston, Mass.

EPITHELIAL hyperplasia and diminished keratinization, usually associated with inflammation, are the principal microscopic changes reported in the gingiva in pregnancy.[1-3] It has not been established whether pregnancy itself produces these changes or whether it simply modifies the gingival response to local irritation so that the epithelial changes are exaggerated.[3, 4] Increased amounts of water-soluble, alcohol-insoluble glycoproteins observed in gingivitis in pregnancy have been attributed to hormonal origin.[5, 6] On the other hand, comparable findings have been associated with gingival inflammation in nonpregnant individuals.[7, 8] It would appear that, in order to determine the effect of pregnancy upon the gingiva, the problem should be studied without the complicating factor of inflammation. Study of the attached gingiva provides such an opportunity.

PROCEDURE

Sixty gingival specimens with and without clinical signs of marginal gingivitis were obtained from 30 patients aged 16 to 32 years, in the second and third trimester of pregnancy. Forty-two specimens, with and without clinical signs of marginal gingivitis, obtained from 21 nonpregnant females in a comparable age group served as controls. Two contiguous rectangular-shaped specimens were taken from each patient on the buccal surface of the maxillary or mandibular premolar and molar areas so as to include the marginal and attached gingivae and, in most cases, the alveolar mucosa (Figs. 1, *A*, and 1, *B*). One specimen was fixed in a solution of 1 per cent trichloracetic acid in 80 per cent alcohol and the other in a solution of 10 per cent formalin in 95 per cent alcohol, buffered to pH 7.0 ± 0.3. Slides in serial sections were prepared from each of these specimens, employing the following staining technics: hematoxylin and eosin; the Barrnett-Seligman technic[9] for protein-bound sulfhydryl and disulfide groups; the Weiss, Tsou, Seligman[10] technic for protein-bound amino groups; and the periodic acid-Schiff technic[11] for glycogen and other water-insoluble, alcohol-insoluble polysaccharides.

From the Ziskin Memorial Research Laboratory, Department of Oral Pathology and Periodontology, Tufts University School of Dental Medicine, Boston, Mass.

This report is from an investigation supported by the Research and Development Division, Office of the Surgeon General, Department of the Army, under Contract No. DA 49-007-MD 706.

Abstract presented at the 36th Annual Meeting, I.A.D.R., Detroit, Mich., March 20-23, 1958.

Received for publication May 7, 1958; revised by authors Sept. 29, 1958.

These groups and compounds were studied because they have been identified with cellular activity and epithelial keratinization,[12-20] features of the gingiva previously reported to be affected by pregnancy.

A.

B.

Fig. 1.—*A*, Rectangular biopsy of marginal and attached gingiva.
B, Typical gingival biopsy of marginal and attached gingiva and portion of alveolar mucosa. (Hematoxylin and eosin; orig. mag. × 28.)

MICROSCOPIC AND HISTOCHEMICAL OBSERVATIONS

All specimens, even those which appeared normal clinically, presented some microscopic signs of inflammation in the marginal gingiva. This varied from a slight cellular infiltration to a pronounced accumulation with increased vascularity and associated degenerative and proliferative changes in the crevicular

epithelium and connective tissue. In cases of severe marginal involvement, the inflammation extended into the attached gingiva. No differences could be detected in the marginal gingiva of the pregnant and nonpregnant patients.

Uninflamed Attached Gingiva, Pregnant and Nonpregnant Groups.—Because of the variation in the configuration of the epithelium of the attached gingiva from specimen to specimen, it was necessary to use a standard for purposes of comparison. The method selected was that of Wentz, Maier, and Orban,[21] with modifications. The epithelium of the attached gingiva of each specimen was classified according to measurements of *ridge length* and *suprapapillary width* made with a Bausch and Lomb micrometer disc, as follows (Fig. 2):

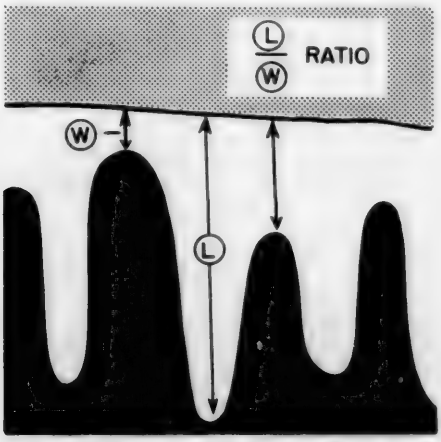


Fig. 2.—L:W ratio used for comparing epithelial configuration in attached gingiva. *L* is the distance from the surface of the epithelium to the tip of the ridge. *W* is the distance from the surface to the base of the epithelium at the tip of the connective tissue papilla.

1. Epithelial ridge length (*L*) was defined as the distance from the epithelial surface to the tip of the ridge.
2. Suprapapillary width (*W*) was defined as the distance from the surface to the base of the epithelium at the tip of the connective tissue papilla.

Averages were computed of the 3 longest epithelial ridges and the narrower of the adjacent suprapapillary zones. Tangentially cut sections were not used.

3. The ratio of $\frac{\text{epithelial ridge length (L)}}{\text{suprapapillary width (W)}}$ was used as the index of the extent of epithelial projection into the underlying connective tissue.

In the uninflamed attached gingiva, the L:W ratio was significantly larger in the pregnant group ($P = 0.05$) (Figs. 3 and 4). This resulted from a relative increase in the length of the ridges, which appeared to be elongated because the suprapapillary width (W) was narrower than in the controls ($P = 0.05$) (Table I). The average L:W ratio in the controls was 4.0 ± 1.2. In the pregnant patients, it was 5.5 ± 1.9. In 31 per cent of the control specimens, the ratio was 4.0 or above, as compared with 84 per cent in the pregnant group. In

58 per cent of the pregnant group, the ratio was 5.0 or above, as compared with only 15 per cent in the controls (Table II). There was an absolute increase in ridge length and over-all thickening of the epithelium in some of the pregnancy specimens, but this did not occur with sufficient frequency to be statistically significant. The ridges in the pregnancy group were thinner than those of the controls.

TABLE I
UNINFLAMED ATTACHED GINGIVA
EPITHELIAL RIDGE LENGTH (L), SUPRAPAPILLARY WIDTH (W), AND L:W OF PREGNANT AND CONTROL GROUPS

	L	W	L:W
Pregnant (19 cases)	385 μ ± 85	80 μ ± 25	5.5 ± 1.9
Control (13 cases)	360 μ ± 55	100 μ ± 20	4.0 ± 1.2

TABLE II
UNINFLAMED ATTACHED GINGIVA (PREGNANT AND CONTROL GROUPS)
DISTRIBUTION OF L:W VALUES

	5.0 OR ABOVE	4.0 OR ABOVE	3.0 OR ABOVE	LESS THAN 3.0
Pregnant (19 cases)	58%	84%	95%	5%
Control (13 cases)	15%	31%	92%	8%

In the control and pregnant patients, the surface of the attached gingiva was either keratinized or parakeratinized. In some specimens both types of surface structure were observed. Surface keratinization was present in only 17 per cent of the pregnant group as compared with 54 per cent of the controls.

Glycogen was present in the epithelium of the uninflamed attached gingiva in 84 per cent (16 out of 19) of the pregnant group and in 30 per cent (4 out of 13) of the control group (Figs. 5 and 6). It was found in the prickle-cell layer of the suprapapillary epithelium or in the core of the elongated ridges, or in both areas. It seldom appeared in the basal layer. The occurrence of glycogen in the epithelial ridges was related to increases in the $\frac{\text{epithelial ridge length}}{\text{suprapapillary width}}$ ratio (Table III).

TABLE III
UNINFLAMED ATTACHED GINGIVA (PREGNANT GROUP)
GLYCOGEN IN CORE OF EPITHELIAL RIDGES AND L:W VALUES

NO. OF SUBJECTS	L:W	NO. OF SUBJECTS SHOWING GLYCOGEN IN CORE	PER CENT
3	2-2.9	0	0
5	4-4.9	2	40
11	5 or over	7	64

Changes occurred in the connective tissue in relation to the elongated epithelial ridges. The basement membrane was thinned. The basement membrane and connective tissue ground substance stained less intensely with the PAS technic, indicative of a reduction in glycogen and carbohydrate-protein complexes (Fig. 6).

Fig. 3.—Section from nonpregnant patient which shows epithelial ridge length:supra-papillary width ratio in uninflamed attached gingiva. (Hematoxylin and eosin; orig. mag. × 200.)

Fig. 4.—Section from pregnant patient which shows altered epithelial ridge length:supra-papillary width ratio in the epithelium of uninflamed attached gingiva. Note relative increase in length and thinning of epithelial ridges. (Hematoxylin and eosin; orig. mag. × 140.)

Fig. 5.—Section from nonpregnant patient which shows absence of glycogen in the epithelium of the attached gingiva. Note the basement membrane and densely staining PAS positive substance in the connective tissue. (Periodic acid-Schiff technic; orig. mag. × 200.)

Fig. 6.—Section from pregnant patient which shows glycogen in the ridges and supra-papillary zones of the epithelium of the attached gingiva. The basement membrane is thinned and barely visible in some areas. The density of the ground substance is reduced. (Periodic acid-Schiff technic; orig. mag. × 140.)

The normal distribution of protein-bound sulfhydryl, disulfide, and amino groups in the epithelium and connective tissue was not affected by pregnancy. In both the pregnant and nonpregnant patients, the protein-bound sulfhydryl and amino groups appeared to be present in greater concentrations in the cells of the longer epithelial ridges, suggesting a relationship between these groups and cellular activity.

Inflamed Attached Gingiva, Pregnant and Nonpregnant Groups.—

In many of the sections, there was evidence of inflammation in the attached gingiva. There were no appreciable differences in the appearance of the inflamed attached gingiva of the pregnant and nonpregnant groups. In both groups, comparable changes occurred associated with inflammation. In the epithelium, there was proliferation, diminished keratinization, an increase in glycogen, sulfhydryls, and amino groups. In the connective tissue, the basement membrane was thinned and the basement membrane and connective tissue ground substance were lighter staining, indicating a diminution in glycogen and carbohydrate-protein complexes.

DISCUSSION

From the findings in the attached gingiva it is possible to evaluate the effects of pregnancy without the complications introduced by inflammation. The major features of the uninflamed attached gingiva in pregnancy were a change in epithelial configuration characterized by a relative lengthening of the epithelial ridges and associated changes in the underlying connective tissue. The latter consisted of a thinning of the basement membrane and a reduction in glycogen and the carbohydrate-protein complexes of the ground substance. It may be theorized that the connective tissue changes in some way facilitated the alteration in epithelial configuration. However, it was not possible to determine the sequence in which these changes occurred.

The change in epithelial configuration was accompanied by diminished surface keratinization. There was also an increased tendency toward the occurrence of glycogen in the epithelium. An increase in glycogen has been described associated with diminished keratinization in the skin of nonpregnant individuals.[15] In the present study, the alteration in epithelial configuration and diminished surface keratinization may be attributable to pregnancy. However, glycogen tends to occur associated with such epithelial changes even in the absence of pregnancy.

It is of interest that the morphologic and histochemical criteria employed in this study revealed no significant differences between the inflamed gingiva of the pregnant and nonpregnant groups. This finding is in conflict with the impression that pregnancy modifies the response of the gingiva to local irritation. Our specimens were obtained from patients whose gingiva either appeared normal clinically or presented slight to moderate marginal gingivitis. The possibility exists that, if there is a modifying effect of pregnancy upon the gingival response to local irritation, it is manifested in patients with clinical disease of greater severity.

CONCLUSION

In a histochemical study of human gingiva, alterations in epithelial configuration characterized by a relative increase in ridge length and diminished surface keratinization were described as changes attributable to pregnancy. Increase in epithelial glycogen, reduction in glycogen and carbohydrate-protein complexes of the connective tissue ground substance, and a thinning of the basement membrane accompanied the morphologic epithelial changes.

Grateful acknowledgment is made to Dr. S. Charles Kasdon for permission to examine the patients at the prenatal clinic of the Boston Dispensary.

REFERENCES

1. Ziskin, D. E., Blackberg, S. H., and Stout, A. P.: The Gingivae During Pregnancy; An Experimental Study and a Histological Interpretation, *Surg., Gynec. & Obst.* **57**: 719, 1933.
2. Ziskin, D. E., and Nesse, G. J.: Pregnancy Gingivitis, History, Classification, Etiology, *Am. J. Orthodont. & Oral Surg.* **32**: 390, 1946.
3. Maier, A. W., and Orban, B.: Gingivitis in Pregnancy, *Oral Surg., Oral Med., & Oral Path.* **2**: 234, 1949.
4. Glickman, I.: Clinical Periodontology, Philadelphia, 1953, W. B. Saunders Co., p. 188.
5. Engel, M. B.: Hormonal Gingivitis, *J. A. D. A.* **44**: 691, 1952.
6. Gans, B. J., Engel, M. B., and Joseph, N. R.: Electrometric Studies of Human Gingiva in Pregnancy, *J. D. Res.* **35**: 566, 1956.
7. Turesky, S., Glickman, I., and Litwin, T.: A Histochemical Evaluation of Normal and Inflamed Human Gingiva, *J. D. Res.* **30**: 792, 1951.
8. Dewar, M. R.: Observations on the Composition and Metabolism of Normal and Inflamed Human Gingivae, *J. Periodont.* **26**: 29, 1955.
9. Barrnett, R. J., and Seligman, A. M.: Histochemical Demonstration of Sulfhydryl and Disulfide Groups of Protein, *J. Nat. Cancer Inst.* **14**: 769, 1954.
10. Weiss, L. P., Tsou, K., and Seligman, A. M.: Histochemical Demonstration of Protein-Bound Amino Groups, *J. Histochem. & Cytochem.* **2**: 29, 1954.
11. Lillie, R. D.: Histopathologic Technic, Philadelphia, 1948, The Blakiston Company, p. 140.
12. Turesky, S., Crowley, J., and Glickman, I.: A Histochemical Study of Protein-Bound Sulfhydryl and Disulfide Groups in Normal and Inflamed Human Gingiva, *J. D. Res.* **36**: 255, 1957.
13. Barron, E. S. G.: Thiol Groups of Biological Importance, *Rec. Adv., Enzymol.* **11**: 201, 1951.
14. Hammett, F. S.: Interpretation of Malignant Growth Based on Chemistry of Cell Division, *Arch. Path.* **8**: 575, 1929.
15. Steiner, K.: A Histochemical Study of Epidermal Glycogen in Skin Diseases, *J. Invest. Dermat.* **24**: 599, 1955.
16. Lobitz, W. C., and Holyoke, J. B.: The Histochemical Response of the Human Epidermis to Controlled Injury; Glycogen, *J. Invest. Dermat.* **22**: 189, 1954.
17. Van Scott, E. J., and Flesch, P.: Sulfhydryl Groups and Disulfide Linkages in Normal and Pathological Keratinization, *Arch. Dermat. & Syph.* **70**: 141, 1954.
18. Giroud, A., and Leblond, C. P.: The Keratinization of Epidermis and Its Derivatives, Especially the Hair as Shown by X-ray Diffraction and Histochemical Studies, *Ann. New York Acad. Sc.* **53**: 613, 1951.
19. A Symposium: Glutathione, New York, 1954, Academic Press, Inc.
20. Bamford, C. H., Elliot, A. X., and Hanby, W. E.: Synthetic Polypeptides, New York, 1956, Academic Press, Inc.
21. Wentz, F. M., Maier, A. W., and Orban, B.: Age Changes and Sex Differences in Clinically "Normal" Gingiva, *J. Periodont.* **23**: 13, 1952.

EMERGENCE OF THE PERMANENT TEETH IN PIMA INDIAN CHILDREN

A Critical Analysis of Method and an Estimate of Population Parameters

ALBERT A. DAHLBERG AND RENÉE M. MENEGAZ-BOCK

Zoller Memorial Dental Clinic and Department of Anthropology, University of Chicago, Chicago, Ill.

THE PROCESS of tooth emergence is traditionally a source of information on the status and progress of physiologic development. Concentrating on the applied aspects of the problem, authors of the studies in the literature are publishing for the practicing dentist, orthodontist, and forensic anthropometrist. The practitioner has found this material useful within broad limits in planning treatment and as a criterion of age. However, both the practitioner and the research worker would profit from a more detailed analysis of the patterns of dental eruption.

Studies of the emergence of the teeth of the Pima Indians showed that replacement of the deciduous teeth with the permanent ones is for them a process which starts later and finishes earlier than in most other populations. These materials also permitted another view of the importance of the second premolar–second molar eruption sequence. Some authors have claimed that the second premolar followed by the second molar is an advanced trait and the reverse, that is, the second molar followed by the second premolar, is a primitive or ancestral sequence. The Pima material supports the rather recent work and discussions by Garn, Koski, and Lewis[1] that the so-called ancestral sequence is common in at least several modern populations. In approaching these and other problems it was found that the parameters, methods, types of studies, and the available data from previous publications had to be subjected to review.

TOOTH EMERGENCE PARAMETER STUDIES

As the number of studies to determine the parameters of the distribution of tooth emergence with age increase, it becomes apparent that a re-evaluation should be made of the basis of comparability of such studies. Clements, Davies-Thomas, and Pickett[2, 3] have taken a step in this direction in their recent study of the age and order of permanent tooth emergence in British children. They point out the necessity for both the assembly of new data and the use of modern methods of analysis. Most of the previous studies based their analyses upon the observation of Klein and associates[4] that the frequencies of erupting teeth at successive ages follow the normal probability

This project was supported by Grant D-165, National Institutes of Health, United States Public Health Service.

Received for publication May 15, 1958; revised by authors Aug. 7, 1958.

function. On this basis, median ages represent the age at which 50 per cent of the children are most likely to have the tooth emerged. The precision of the statistical methods for analyzing integral responses of the normal probability function has been improved since Klein's work by the development of probit analysis.[5] This maximum likelihood method of fitting the data makes it possible to specify the statistical error of the estimates (see "Methods").

Some authors,[6, 7, 8] referring to Broca,[9] take serious objection to the reporting of population parameters of tooth emergence in "medians" rather than "means" and to the current concepts and methods of curve fitting. Actually, the assumption of normality is questioned when the upper asymmetry of the distribution is emphasized and a decision made to smooth the curve by eye. In choosing the mean as the preferred statistic to report, no attention is paid to the fact that the mean of an unknown distribution is meaningless! Precisely what the upper skewness signifies if it appears shall be discussed later. Immediately pertinent to a discussion of methods in dental eruption studies is that the mean of an unknown distribution does not tell very much about the distribution. If the worker is confident that the underlying distribution of the data is other than normal, simply substituting the mean for the median is not adequate. To describe a distribution which probably depends on 3 parameters, the worker must derive the function which describes his non-normal distribution or use one of Pearson's curves described uniquely by 4 moments, measuring central tendency, dispersion, skewness, and kurtosis.[10]

The important question to ask when confronted with biologic data which do not seem to fit the assumed normality of the underlying distribution is: "Does firm evidence of departure from normality exist?" This is certainly the question to ask when some large sample studies have already appeared in the literature which do not show any departure from normality.[2, 4, 11]

If the data appear to be skewed, it ought to be examined for cases of extractions, impactions, and other pathologic conditions in order to determine the obviously distoring phenomena operating in the later age groups to disturb the trend established in the earlier age groups. The answer to the above question must also take account of the size of the sample taken, the sampling method, and the possibility of distinctly different biologic factors acting in the "aberrant" age groups. It is possible that in the case of tooth emergence even a sufficiently large and representative sample of children will show the later emerging teeth skewed toward later eruption. It is conceivable that caries experience, eating habits, occlusal architecture, and delayed puberty are among the conditions probably responsible for such a picture. If this is the case, then it is necessary that the "exceptional" not be permitted to distort the evaluation of the "usual" (i.e., the "normal"). Each provides information of interest in its own right which should not be masked by the analysis. Data with skewed curves in the range for later age groups should be examined as suggested above and, if legitimate evidence exists for doing so, the data should be censored at the top. It must be noted here that there is as yet no

good evidence in the literature which would uphold the assumption of an essentially non-normal distribution of tooth emergence. It could be argued that Chi squared is too crude a test to detect slight deviations at the ends of the curves. If deviations are consistently found, the distribution of age of tooth emergence in the population could be considered as the result of two overlapping normal distributions[10]; the major one reflecting the dental development of the greater number of children, the minor one reflecting the dental development of the few children exhibiting delayed or inhibited dental development.

Both the clinician and the physical anthropologist require that the "usual" be reported separately from the "exceptional"; the clinician, so that his expectations for treatment will correspond more closely to what is likely to occur in the majority of his patients; the research worker, so that the basis for comparisons between populations can be as exactly defined as the data permit.

CROSS-SECTIONAL VERSUS LONGITUDINAL STUDIES

Bearing on the question of comparability of data in the literature is the evaluation of cross-sectional versus longitudinal studies. The means from longitudinal studies should be comparable directly to the medians derived from cross-sectional studies, given proper sampling, if the underlying distribution is in fact normal. It is very difficult, however, to match in a longitudinal study the large numbers achievable in a cross-sectional study. This is one reason a cross-sectional study in which the data are properly analyzed has a better chance of representing the population more faithfully. A more important reason for considering cross-sectional studies of greater reliability is the bias in a longitudinal study toward later estimates. No longitudinal study in the literature has had one half the examination intervals subtracted from the ages at which the teeth are first seen. Since the observer can see the tooth only *after* it erupts and never before, he is always estimating upward. The proper correction for this phenomenon is to subtract from each subject's age of eruption one half the examination interval on the assumption of uniformly distributed emergence during that interval. The usual procedure in published longitudinal studies of dental eruption is to group to the nearest endpoint of the examination interval. Therefore, approximately one half of the ages have additions and one half have subtractions. The additions serve to increase even more an already late age estimate. This bias becomes larger as the interval between examinations increases. A comparison of cross-sectional with longitudinal studies reviewed here certainly indicates a tendency in the direction of later estimations for all teeth by the longitudinal studies. Table I shows an average difference of half a year older for emergence in the longitudinal studies as compared to the horizontal ones. For these reasons, the more stable results of a large-scale cross-sectional study are often more useful to the orthodontist (as a basis for timing treatment), to the biologist (as an indicator of stage of maturation), and to the forensic anthropometrist (as one of the criteria of age).

TABLE I
A COMPARISON OF ESTIMATES OF AGE OF ERUPTION BY LONGITUDINAL VERSUS CROSS-SECTIONAL METHODS

GROUP	SOURCE REFERENCE	LONGITUDINAL METHOD													
		MAXILLARY TEETH							MANDIBULAR TEETH						
		$^1I^1$	$^2I^2$	$^1C^1$	$^1Pm^1$	$^2Pm^2$	$^1M^1$	$^2M^2$	$_1I_1$	$_2I_2$	$_1C_1$	$_1Pm_1$	$_2Pm_2$	$_1M_1$	$_2M_2$
						MALES									
Negro															
Am. Negro	Steggerda & Hill[18]	7.77	8.45	11.74	10.82	11.92	6.79	12.64	6.95	7.94	10.99	10.86	11.48	6.97	12.33
Am. Indian															
Maya	Steggerda & Hill[18]	8.35	9.30	11.79	10.29	11.63	6.88	12.49	7.41	8.40	11.16	11.14	11.99	6.76	11.86
Navajo	Steggerda & Hill[18]	7.65	8.84	11.07	10.09	10.90	6.68	11.56	6.80	7.72	10.26	10.22	11.18	6.33	11.62
Caucasoid															
American	Steggerda & Hill[18]	7.87	9.16	11.83	10.72	11.46	7.04	13.05	6.93	8.51	10.94	11.15	11.86	6.98	12.41
American	Hellman[15]	7.33	8.60	12.02	11.17	12.21	6.75	12.96	6.29	7.55	11.04	11.09	12.32	6.82	12.59
American	Fulton & Price[12]	7.26	8.42	11.38	10.42	11.12	6.63	12.09	6.54	7.54	10.75	11.09	11.80	6.32	11.83
Caucasoid English	Stones, Lawton, Bransby, & Hartley[14]	8.10	8.85	12.24	10.93	11.38	6.72	12.43	6.85	8.12	11.41	11.40	12.12	6.91	12.16
Mean		*7.76*	*8.80*	*11.72*	*10.63*	*11.52*	*6.78*	*12.46*	*6.82*	*7.97*	*10.94*	*10.99*	*11.82*	*6.73*	*12.11*
		CROSS-SECTIONAL METHOD													
						MALES									
Negro															
Zulu	Suk[20]	5.98	6.98	10.17	10.11	10.66	5.26	11.36	5.47	5.96	9.63	10.11	10.75	5.23	11.04
Pima	Dahlberg	7.83	8.74	11.66	10.08	11.33	5.98	11.67	6.26	7.65	10.78	10.43	11.39	5.89	11.29
American	Cattell[11]	7.33	8.42	11.50	10.33	11.08	6.33	12.16	6.25	7.58	10.66	10.58	11.33	6.16	11.66
American	Cohen[20]	7.1	8.6	11.5	10.2	10.7	6.4	12.8	6.4	7.5	10.3	10.8	11.4	6.4	11.8
American	Klein, Palmer, & Kramer[4]	7.49	8.62	11.80	10.42	11.18	6.64	12.70	6.50	7.64	10.70	10.75	11.45	6.44	12.20
English	Ainsworth[17]	7.42	8.81	11.73	9.96	10.89	6.34	12.33	6.49	7.72	10.80	10.86	11.80	6.24	11.86
English	Clements, Davies-Thomas & Pickett[2]	7.01	8.18	11.46	10.41	11.52	6.11	11.97	6.08	7.30	10.51	11.35	12.32	6.14	11.41
Mean		*7.17*	*8.34*	*11.40*	*10.22*	*11.05*	*6.15*	*12.14*	*6.21*	*7.34*	*10.48*	*10.70*	*11.49*	*6.07*	*11.61*
Difference—Males (Longitudinal Method)		.59	.46	.32	.41	.47	.63	.32	.61	.63	.46	.29	.33	.66	.50

TABLE I—CONT'D

GROUP	SOURCE REFERENCE	$^1I^1$	$^2I^2$	$^1C^1$	$^1Pm^1$	$^2Pm^2$	$^1M^1$	$^2M^2$	$_1I_1$	$_2I_2$	$_1C_1$	$_1Pm_1$	$_2Pm_2$	$_1M_1$	$_2M_2$
		LONGITUDINAL METHOD													
		MAXILLARY TEETH							MANDIBULAR TEETH						
		FEMALES													
Am. Negro	Steggerda & Hill[18]	7.13	8.31	10.39	10.07	10.97	6.90	11.85	6.28	7.19	9.73	10.23	10.77	6.33	11.43
Am. Indian															
Maya	Steggerda & Hill[18]	8.27	8.63	10.89	9.96	10.92	6.69	12.09	7.15	8.09	10.32	10.24	11.16	6.68	11.49
Navajo	Steggerda & Hill[18]	7.71	8.66	10.68	9.88	11.38	6.61	11.43	6.48	7.44	9.81	10.18	10.47	6.43	11.23
Caucasoid															
American	Steggerda & Hill[18]	7.59	8.78	11.41	10.52	11.33	6.97	12.62	6.58	8.00	10.22	10.63	11.52	6.59	11.86
American	Hellman[15]	7.24	8.17	11.74	10.83	11.94	6.89	12.93	6.26	7.43	10.26	10.66	11.74	6.70	12.61
American	Fulton & Price[12]	7.00	7.87	10.55	9.91	10.46	6.32	11.59	6.21	6.92	9.66	10.08	10.79	6.04	10.91
Caucasoid English	Stones, Lawton, Bransby, & Hartley[14]	7.67	8.66	12.01	10.47	11.11	6.60	12.11	6.81	8.05	10.67	11.43	12.01	6.57	12.21
Mean		*7.52*	*8.54*	*11.10*	*10.23*	*11.16*	*6.71*	*12.09*	*6.54*	*7.59*	*10.10*	*10.49*	*11.31*	*6.48*	*11.68*
		CROSS-SECTIONAL METHOD													
		FEMALES													
Zulu	Suk[20]	6.18	7.14	9.72	9.76	10.06	5.77	10.92	5.85	6.23	9.12	9.76	10.24	5.49	10.61
Pima	Dahlberg	7.47	8.34	10.94	9.63	10.73	5.80	11.38	6.15	7.32	9.66	9.87	10.73	5.43	10.80
American	Cattell[11]	7.08	8.00	11.08	9.92	10.92	6.16	12.08	6.08	7.25	9.66	10.08	11.08	6.00	11.42
American	Cohen[20]	6.9	7.9	10.7	9.9	10.7	6.1	12.2	6.1	7.0	9.6	10.1	10.8	5.9	11.6
American	Klein, Palmer, & Kramer[4]	7.20	8.15	11.05	10.00	10.82	6.54	12.40	6.19	7.31	9.85	10.20	11.00	6.12	11.90
English	Ainsworth[17]	7.20	8.37	11.20	9.77	10.72	6.12	12.07	6.23	7.50	9.90	10.3	11.21	5.95	11.52
English	Clements, Davies-Thomas & Pickett[2]	6.62	7.82	10.67	9.79	11.06	5.94	11.50	5.77	7.01	9.41	10.53	11.64	5.84	11.18
Mean		*6.95*	*7.96*	*10.77*	*9.82*	*10.72*	*6.06*	*11.79*	*6.05*	*7.09*	*9.60*	*10.13*	*10.96*	*5.82*	*11.39*
Females, longitudinal minus cross-sectional:		.57	.58	.33	.41	.44	.65	.30	.49	.50	.50	.36	.25	.66	.39

Fulton and Price[12] claim that only a longitudinal study demonstrates the 'batching" of eruption times of the teeth. However, a glance at the accompanying graphs of population median ages of tooth emergence (see Figs. 2 and 3) shows that the early erupting set and the later erupting set are distinctly separated. Curves of tooth emergence for individual children give some insight into individual patterns of development. This understanding is of value to the practicing dentist although he should also know the statistics of the population from which his patients are drawn. It is of less value to the physical anthropologist interested in racial comparisons.

TOOTH EMERGENCE DATA IN PIMA INDIANS

The Pima Indian permanent tooth emergence data consist of observations made on dental casts of Pima Indian children ranging in age from 3 to 14 years and 11 months. The casts are part of the collection of Pima Indian materials in the Zoller Laboratory of Dental Anthropology at the University of Chicago. They were collected over a period starting in 1946 and continuing through 1957. Readings from the casts were charted for the whole mouth of each child and each tooth classified as present or absent. Unerupted and extracted teeth were classified as not present. Extractions among this group of Pima children are considered to be too few to distort the count of unerupted teeth. Teeth were classified as present if any part of the tooth had pierced the gum.

TABLE II
AGE DISTRIBUTION OF PIMA INDIAN SUBJECTS

MIDPOINT AGE CLASS		CODE	FEMALE (NUMBER)	MALE (NUMBER)
3.25	=	1	1	0
3.75		2	1	2
4.25		3	3	2
4.75		4	2	3
5.25		5	7	6
5.75		6	8	8
6.25		7	40	36
6.75		8	56	49
7.25		9	37	53
7.75		10	33	27
8.25		11	31	25
8.75		12	31	25
9.25		13	30	29
9.75		14	26	30
10.25		15	30	26
10.75		16	29	32
11.25		17	15	20
11.75		18	24	22
12.25		19	16	14
12.75		20	17	13
13.25		21	13	20
13.75		22	18	15
14.25		23	13	6
14.75		24	6	7
Total			487	470

Table II shows the distribution of subjects by age group. The children were unselected except by attendance at the Federal Indian and private schools on the Pima Indian Reservations in Arizona. The 3-, 4-, and 5-year-old

children were prospective first graders seen at home in an effort to fill in the younger age groups. The 470 boys and 487 girls represent approximately 37 per cent of the Pima Indian child population in the age range of our study. The siblings included in the sample are considered to be too few to bias the results. In the interest of racial comparisons it should be noted that the Pima Indian population is 97.4 per cent full blood according to government statistics.

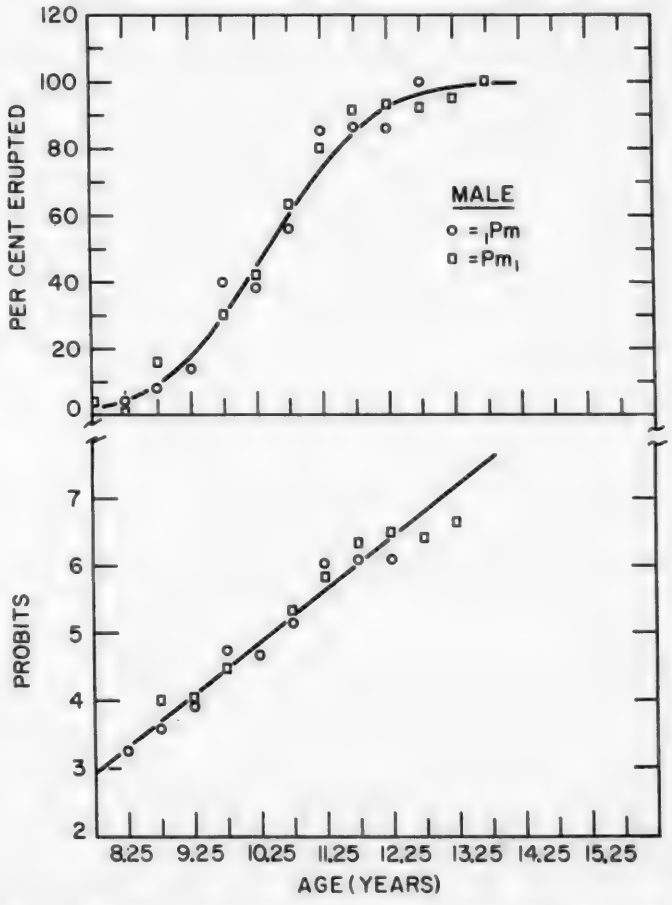


Fig. 1.—The probit transformation. *Upper*—the proportion of boys in each age group having the mandibular first right and left premolars erupted. *Lower*—the proportions converted to normal equivalent deviates plus five (i.e., probits).

PROBIT ANALYSIS OF DATA

The data were punched on IBM cards and counted on the IBM 407. They were then graphed and fitted to a normal probability function by the method of probits.[5] The Pima Indian material shows no departure from the fitted normal curve when tested by Chi squared. In fitting quantal response (all

or none) data, such as tooth eruption classified as present versus not present, to a normal probability function the best method is that of maximum likelihood, in this case probit analysis. The computations are based on the observed probabilities of finding the tooth erupted at various ages. These proportions are converted into probits, i.e., normal equivalent deviates plus 5, in order to rectify their relationship to age. This transformation (Fig. 1) of percentage to normal equivalent deviates, changes the sigmoid curve of cumulative frequency to a straight line when the probits are plotted against age. The observed points are expected to fall on a straight line within the limits of sampling error. A weighted regression analysis is then carried out in terms of working probits to determine the goodness of fit of the points observed to the line drawn. If the Chi-square test for the goodness of fit shows no significant departure from normality, it is concluded that the data under examination are taken from a homogeneous population. The procedure may be repeated if a better fit to the line is required. The fitted line is then taken to represent the distribution of the per cent of eruption with age in the population sampled. Knowing this distribution, the expected per cent for tooth emergence at any age can be best estimated. It is from this fitted regression line that the median eruption age and the standard error of the median for each tooth, as well as the standard deviation of the distribution, are determined. The median is that point at which 50 per cent of the subjects are most likely to show the tooth erupted.

RESULTS

The Pima Indian material provides information on the emergence ages for 28 of the 32 permanent teeth. No erupted third molars were observed. Table II shows the age distribution and the number of boys and girls in the study.

TABLE III
MEDIAN AGES OF ERUPTION IN PIMA INDIAN CHILDREN, STANDARD ERRORS OF THE MEDIAN AND STANDARD DEVIATIONS OF THE DISTRIBUTION (YEARS)

	MAXILLARY TEETH							MANDIBULAR TEETH						
	$^{1}I^{1}$	$^{2}I^{2}$	$^{1}C^{1}$	$^{1}Pm^{1}$	$^{2}Pm^{2}$	$^{1}M^{1}$	$^{2}M^{2}$	$_{1}I_{1}$	$_{2}I_{2}$	$_{1}C_{1}$	$_{1}Pm_{1}$	$_{2}Pm_{2}$	$_{1}M_{1}$	$_{2}M_{2}$
Boys														
Med.	7.83	8.74	11.66	10.08	11.33	5.98	11.67	6.26	7.65	10.78	10.43	11.39	5.89	11.29
S.E.	.07	.09	.14	.12	.14	.09	.14	.10	.12	.13	.12	.15	.09	.14
S.D.	.71	.75	1.41	1.28	1.36	.77	1.21	.89	1.21	1.28	1.29	1.45	.67	1.25
Girls														
Med.	7.47	8.34	10.94	9.63	10.73	5.80	11.38	6.15	7.32	9.66	9.87	10.73	5.43	10.80
S.E.	.08	.08	.16	.12	.14	.10	.13	.09	.09	.11	.13	.14	.16	.12
S.D.	.69	.98	1.59	1.23	1.34	.79	1.29	.75	1.02	1.25	1.30	1.46	.99	1.05

Table III records the median emergence age and the standard error of the median for pairs of corresponding teeth, upper and lower jaws for each sex. The probable sequence of tooth emergence in the Pima according to the population median emergence ages is set out in Table IV. The order is different in the maxilla but the same in the mandible for boys and girls. The order

is different in the upper and lower jaws. The data of Clements and coworkers[3] on English children are shown for comparison. An interesting aspect of the order of tooth emergence which cannot be discussed here is the correlation found by Lo and Moyers[13] between sequence of tooth emergence and patterns of occlusion.

TABLE IV
ORDER OF TOOTH EMERGENCE IN PIMA AND ENGLISH CHILDREN ACCORDING TO CALCULATED POPULATION MEDIANS (IN YEARS)

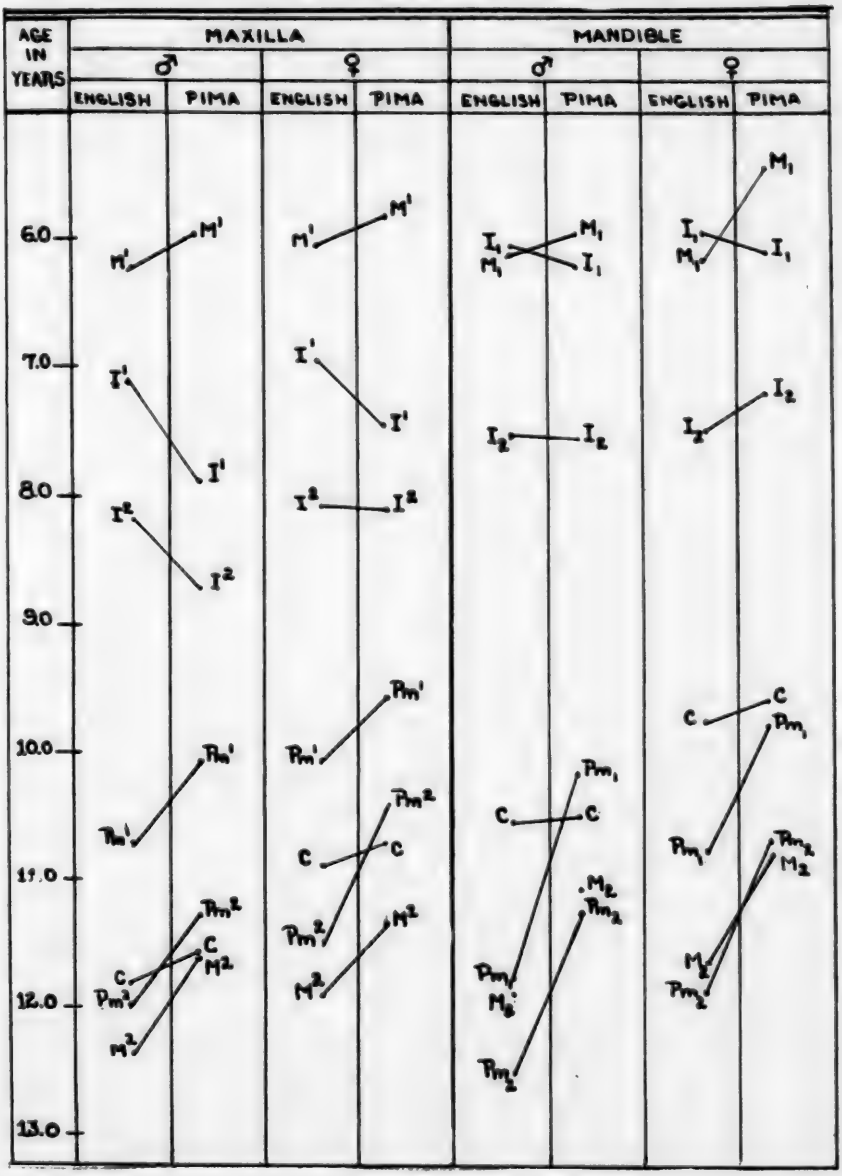


Table V lists the differences between the median emergence ages of the individual members of the tooth pairs on the right and left sides of the same jaw. There is no consistent bias in the sign of these differences, nor does inspection indicate any statistical significance in the magnitude of the differences. This agrees with the results of the majority of tooth emergence studies. Stones, Lawton, Bransby, and Hartley,[14] however, conclude from their data that the right side takes preference in order for the incisors, canines, and premolars. Their small sample size may account for their results.

TABLE V
DIFFERENCES BETWEEN THE MEDIAN AGES OF EMERGENCE, AND STANDARD ERROR OF THE DIFFERENCE OF THE TOOTH PAIRS, RIGHT MINUS LEFT, PIMA INDIAN CHILDREN

	MAXILLARY TEETH						
	$^{1}I^{1}$	$^{2}I^{2}$	$^{1}C^{1}$	$^{1}Pm^{1}$	$^{2}Pm^{2}$	$^{1}M^{1}$	$^{2}M^{2}$
Boys	−.02 ± .10	+.07 ± .13	−.11 ± .20	+.15 ± .17	−.01 ± .19	−.09 ± .13	.00 ± .20
Girls	−.09 ± .11	+.04 ± .11	+.29 ± .22	−.22 ± .17	+.02 ± .19	+.02 ± .14	−.10 ± .19
	MANDIBULAR TEETH						
	$_{1}I_{1}$	$_{2}I_{2}$	$_{1}C_{1}$	$_{1}Pm_{1}$	$_{2}Pm_{2}$	$_{1}M_{1}$	$_{2}M_{2}$
Boys	−.02 ± .15	+.03 ± .16	−.16 ± .18	+.06 ± .16	−.06 ± .26	+.02 ± .13	−.15 ± .19
Girls	−.02 ± .13	+.04 ± .13	−.08 ± .16	−.25 ± .18	+.24 ± .19	+.36 ± .21	+.10 ± .17

Differences in median time of entry between homologous teeth in the upper and lower jaws are shown in Table VI for boys and girls, separately. All mandibular teeth erupt before their maxillary counterparts except for the first mandibular premolar. This break in the pattern of mandibular precedence also occurs in the sample of English school children studied by Clements and associates.[2, 3] The biologic basis for the later eruption of the first lower premolar over the first upper premolar is unknown and cannot even be speculated upon at the present time. A similar inversion of pattern occurs in the eruption of deciduous teeth where the maxillary lateral incisor precedes the mandibular lateral incisor.

TABLE VI
DIFFERENCES BETWEEN THE MEDIAN AGES OF EMERGENCE AND STANDARD ERROR OF THE DIFFERENCE OF THE HOMOLOGOUS TEETH, MAXILLARY MINUS MANDIBULAR, PIMA INDIAN CHILDREN

	RIGHT						
	1I	2I	C	1Pm	2Pm	1M	2M
Boys	1.57 ± .11	1.11 ± .14	0.95 ± .20	−.31 ± .17	−.04 ± .26	.03 ± .14	.46 ± .19
Girls	1.38 ± .12	1.02 ± .12	1.46 ± .21	−.22 ± .16	−.11 ± .25	.25 ± .16	.48 ± .18
	LEFT						
	1I	2I	C	Pm1	Pm2	M1	M2
Boys	1.57 ± .12	1.07 ± .15	0.68 ± .18	−.40 ± .16	−.09 ± .19	.14 ± .12	.31 ± .19
Girls	1.35 ± .11	1.02 ± .12	1.09 ± .17	−.25 ± .19	.11 ± .18	0.59 ± .21	0.68 ± .17

TABLE VII
DIFFERENCES BETWEEN THE SEXES IN MEDIAN AGES OF TOOTH EMERGENCE AND STANDARD ERROR OF THE DIFFERENCES, BOYS MINUS GIRLS, PIMA INDIAN CHILDREN

	1I1	2I2	1c1	1Pm1	2Pm2	1M1	2M2
Maxilla	.36 ± .11	.40 ± .13	.62 ± .21	.45 ± .17	.60 ± .20	.18 ± .13	.29 ± .19
Mandible	.11 ± .13	.33 ± .15	1.12 ± .17	.56 ± .18	.66 ± .20	.46 ± .18	.49 ± .18

A comparison by sex of the median emergence ages of the corresponding pairs of teeth is given in Table VII. The differences shown in the table affirm the known sexual bias for earlier physical development in girls. Note that without exception all of the male medians are above those for females. Fig. 2 depicts the relations enumerated in Tables III, IV, V, VI, and VII. Table VIII indicates how universal is the occurrence of this sex distinction in age of tooth emergence. Chief of the few exceptions is the reversal Suk's Zulu data show for the first molar. If Suk's data[19] were supported by other studies on Negroes or if Hellman's[15] analysis and interpretation of it were more reliable, the claim of earlier development of Zulu boys over Zulu girls might be more convincing. As the case stands, it is easier to doubt the reliability of the data than to believe that the Zulu boys and girls are exceptional in this respect.

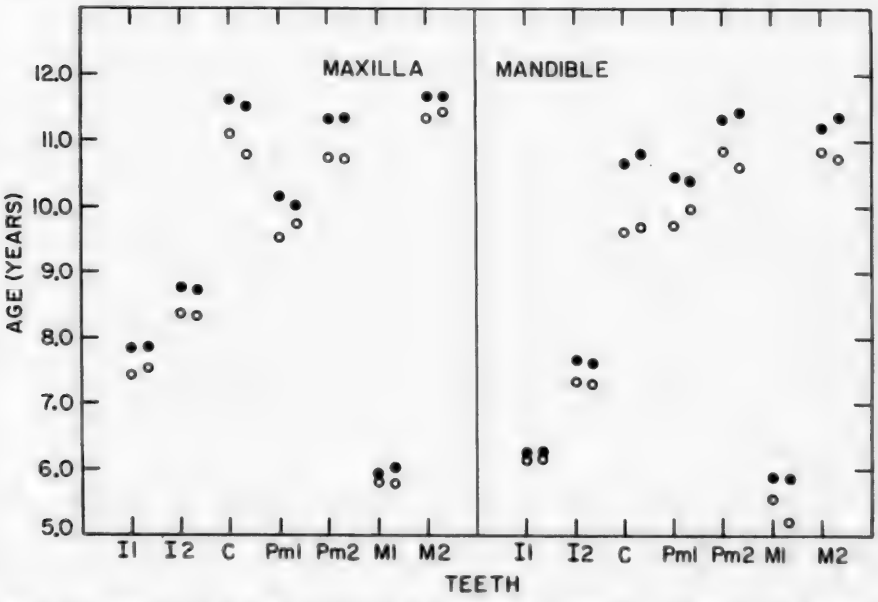


Fig. 2.—Median ages of tooth emergence in Pima Indian children. ● = Boys; O = Girls.

DISCUSSION

To test the significance of the difference between the medians in the above tables, it is the usual practice, but statistically unsound, [10] to compute a series of "t" tests. Because emergence data are made up of multiple measures on the same subject, they do not meet the requirement of independence among the variables that a valid series of "t" tests requires. To illustrate: each child's chart contains data on 28 tooth positions. He is included in only one age group but his data are included in 28 regression analyses. The medians computed from these 28 fitted regression lines are each influenced by a piece

TABLE VIII

ESTIMATES OF AGE OF TOOTH EMERGENCE, MEDIANS OR MEANS (IN YEARS), ± ONE STANDARD DEVIATION, IN NEGRO, AMERICAN INDIAN, AND CAUCASOID AMERICAN AND ENGLISH POPULATIONS

GROUP	SOURCE	METHOD*	RANGE OF SIZE OF N	MAXILLARY TEETH							MANDIBULAR TEETH						
				$^{1}I^{1}$	$^{2}I^{2}$	$^{1}C^{1}$	$^{1}Pm^{1}$	$^{2}Pm^{2}$	$^{1}M^{1}$	$^{2}M^{2}$	$_{1}I_{1}$	$_{2}I_{2}$	$_{1}C_{1}$	$_{1}Pm_{1}$	$_{2}Pm_{2}$	$_{1}M_{1}$	$_{2}M_{2}$
MALES																	
Am. Negro	Steggerda & Hill[18]	L, m	From 9 to 50	7.77 ±.66	8.45 .81	11.74 .97	10.82 1.09	11.92 1.03	6.79 .79	12.64 .92	6.95 ±.59	7.94 .76	10.99 .94	10.86 .87	11.48 .90	6.97 .70	12.33 1.02
Zulu	Suk[19]	C, med.	492	5.98 ±1.07	6.98 1.40	10.17 1.55	10.11 1.35	10.66 1.19	5.26 .69	11.36 1.21	5.47 ±.73	5.96 1.04	9.63 1.31	10.11 1.38	10.75 1.28	5.23 .71	11.04 1.17
Maya	Steggerda & Hill[18]	L, m	25 to 67	8.35 ±.67	9.30 .88	11.79 1.13	10.29 1.01	11.63 1.03	6.88 .55	12.49 .99	7.41 ±.67	8.40 .53	11.16 .90	11.14 1.00	11.99 1.08	6.76 .62	11.86 1.07
Navajo	Steggerda & Hill[18]	L, m	23 to 89	7.65 ±.67	8.84 .81	11.07 .90	10.09 .87	10.90 .89	6.68 .69	11.56 .92	6.80 ±.70	7.72 .66	10.26 .75	10.22 .87	11.18 .91	6.33 .58	11.62 .95
Pima	Dahlberg	C, med.	470	7.83 ±.71	8.74 .75	11.66 1.41	10.08 1.28	11.33 1.36	5.98 .77	11.67 1.21	6.26 ±.89	7.65 1.21	10.78 1.28	10.43 1.29	11.39 1.45	5.89 .67	11.29 1.25
American	Steggerda & Hill[18]	L, m	38 to 154	7.87 ±.68	9.16 .62	11.83 .81	10.72 .98	11.46 .11	7.04 .25	13.05 .95	6.93 ±.32	8.51 .54	10.94 .88	11.15 1.01	11.86 1.12	6.98 .31	12.41 .88
American	Hellman[15]	L, m	452	7.33 ±1.07	8.60 1.14	12.02 1.67	11.17 1.39	12.21 1.62	6.75 .84	12.96 1.48	6.29 ±.77	7.55 1.06	11.04 1.05	11.09 1.23	12.32 1.50	6.82 .82	12.59 1.09
American	Fulton & Price[12]	L, m	100	7.26 ±.96	8.42 1.00	11.38 .96	10.42 1.39	11.12 1.60	6.63 .75	12.09 1.46	6.54 ±.75	7.54 .89	10.75 1.06	11.09 1.56	11.80 1.53	6.32 .89	11.83 1.28
American	Cattell[11]	C, med.	3,863	7.33 ±.74	8.42 .79	11.50 1.20	10.33 1.59	11.08 1.31	6.33 .79	12.16 1.13	6.25 ±.68	7.58 .79	10.66 1.02	10.58 1.36	11.33 1.54	6.16 .79	11.66 .86
American	Cohen[20]	C, med.	1,424 (approx.)	7.1 ±.82	8.6 .82	11.5 1.36	10.2 1.36	10.7 1.37	6.4 .54	12.8 1.09	6.4 ±.54	7.5 .82	10.3 1.09	10.8 1.36	11.4 2.08	6.4 .68	11.8 1.36
American	Klein et al.[4]	C, med.	2,232	7.49 ±.75	8.62 1.10	11.80 1.42	10.42 1.50	11.18 1.68	6.64 .75	12.70 1.18	6.50 ±.74	7.64 .80	10.70 1.15	10.75 1.42	11.45 1.77	6.44 .75	12.20 1.20
English	Stones et al.[14]	L, m	30 to 108	8.10 ±1.31	8.85 .92	12.24 1.20	10.93 1.52	11.38 1.55	6.72 .50	12.43 1.06	6.85 ±1.26	8.12 .93	11.41 1.25	11.40 1.47	12.12 1.53	6.91 .51	12.16 1.01
English	Ainsworth[17]	C, med.	2,000 (approx.)	7.42 ±.69	8.81 .94	11.73 1.12	9.96 1.42	10.89 1.50	6.34 .68	12.33 1.06	6.49 ±.69	7.72 .71	10.80 1.09	10.86 1.33	11.80 1.50	6.24 .69	11.86 1.09
English	Clements et al.[2]	C, med.	1,427	7.01	8.18	11.46	10.41	11.52	6.11	11.97	6.08	7.30	10.51	11.35	12.32	6.14	11.41
New Zealand	Leslie[16]	C, med.	1,427	7.26 ±.80	8.32 .90	11.40 1.12	11.01 1.37	11.74 1.29	6.47 .78	12.47 1.33	6.38 ±.59	7.42 .83	10.78 .93	11.34 1.42	12.18 1.44	6.46 .76	11.89 1.13

*L = longitudinal; C = cross-sectional; m = mean; med. = median.

TABLE VIII—CONT'D

GROUP	SOURCE	METHOD*	RANGE OF SIZE OF N	MAXILLARY TEETH							MANDIBULAR TEETH						
				$^1I^1$	$^2I^2$	$^1C^1$	$^1Pm^1$	$^2Pm^2$	$^1M^1$	$^2M^2$	$_1I_1$	$_2I_2$	$_1C_1$	$_1Pm_1$	$_2Pm_2$	$_1M_1$	$_2M_2$
				FEMALES													
Am. Negro	Steggerda & Hill[18]	L, m	8 to 50	7.13	8.31	10.39	10.07	10.97	6.90	11.85	6.28	7.19	9.73	10.23	10.77	6.33	11.43
				±.60	.88	.85	.79	.81	.45	.75	±.79	.70	.92	.87	.90	.62	.99
Zulu	Suk[19]	C, med.	516	6.18	7.14	9.72	9.76	10.06	5.77	10.92	5.85	6.23	9.12	9.76	10.24	5.49	10.61
				±.93	1.25	1.44	1.27	1.23	.57	1.38	±.73	1.03	1.07	1.12	1.32	.75	1.49
Maya	Steggerda & Hill[18]	L, m	13 to 55	8.27	8.63	10.89	9.96	10.92	6.69	12.09	7.15	8.09	10.32	10.24	11.16	6.68	11.49
				±.85	.76	1.00	.94	.94	.70	1.01	±.78	.81	.81	.87	.90	.76	1.10
Navajo	Steggerda & Hill[18]	L, m	23 to 88	7.71	8.66	10.68	9.88	11.38	6.61	11.43	6.48	7.44	9.81	10.18	10.47	6.43	11.23
				±.57	.60	.87	.87	1.12	.55	.78	±.54	.52	.86	.75	.82	.52	.95
Pima	Dahlberg	C, med.	487	7.47	8.34	10.94	9.63	10.73	5.80	11.38	6.15	7.32	9.66	9.87	10.73	5.43	10.80
				±.69	.98	1.59	1.23	1.34	.79	1.29	±.75	1.02	1.25	1.30	1.46	.99	1.05
American	Steggerda & Hill[18]	L, m	24 to 127	7.59	8.78	11.41	10.52	11.33	6.97	12.62	6.58	8.00	10.22	10.63	11.52	6.59	11.86
				±.75	.76	.92	1.07	1.09	.62	.94	±.37	.67	.76	.88	1.15	.37	.90
American	Hellman[15]	L, m	590	7.24	8.17	11.74	10.83	11.94	6.89	12.93	6.26	7.43	10.26	10.66	11.74	6.70	12.61
				±.64	1.04	1.89	1.15	1.98	.82	1.74	±.87	.76	1.07	1.04	1.67	.90	1.79
American	Fulton & Price[12]	L, m	100	7.00	7.87	10.55	9.91	10.46	6.32	11.59	6.21	6.92	9.66	10.08	10.79	6.04	10.91
				1.06	.96	1.60	1.56	1.70	.89	1.49	±.75	1.00	1.60	1.39	1.95	.67	1.63
American	Cattell[11]	C, med.	3,826	7.08	8.00	11.08	9.92	10.92	6.16	12.08	6.08	7.25	9.66	10.08	11.08	6.00	11.42
				±.68	.79	1.07	1.31	1.42	.57	1.13	±.52	.68	.91	1.13	1.42	.45	1.07
American	Cohen[20]	C, med.	1,424 (approx)	6.9	7.9	10.7	9.9	10.7	6.1	12.2	6.1	7.0	9.6	10.1	10.8	5.9	11.6
				±.54	.95	1.37	1.09	1.36	.68	1.09	±.41	.95	.95	1.09	1.37	.68	1.09
American	Klein et al.[4]	C, med.	2,153	7.20	8.15	11.05	10.00	10.82	6.54	12.40	6.19	7.31	9.85	10.20	11.00	6.12	11.90
				±.75	.94	1.37	1.40	1.60	.70	1.40	±.70	.75	1.15	1.41	1.70	.87	1.55
English	Stones et al.[14]	L, m	26 to 65	7.67	8.66	12.01	10.47	11.11	6.60	12.11	6.81	8.05	10.67	11.43	12.01	6.57	12.21
				±.97	.98	1.44	1.44	1.47	.67	1.03	±.96	.94	1.46	1.46	1.52	.65	1.06
English	Ainsworth[17]	C, med.	2,000 (approx)	7.20	8.37	11.20	9.77	10.72	6.12	12.07	6.23	7.50	9.90	10.36	11.21	5.95	11.52
				±.63	.94	1.12	1.13	1.33	.68	1.03	±.65	.72	.95	1.22	1.46	.68	1.03
English	Clements et al.[2]	C, med.	1,365	6.62	7.82	10.67	9.79	11.06	5.94	11.50	5.77	7.01	9.41	10.53	11.64	5.84	11.18
New Zealand	Leslie[16]	C, med.	1,335	6.83	7.86	10.82	10.52	11.24	6.38	12.20	6.19	7.16	9.74	10.54	11.73	6.30	11.36
				±.69	.61	1.02	.99	1.20	.78	1.28	±.60	.62	.94	1.18	1.35	.59	1.47

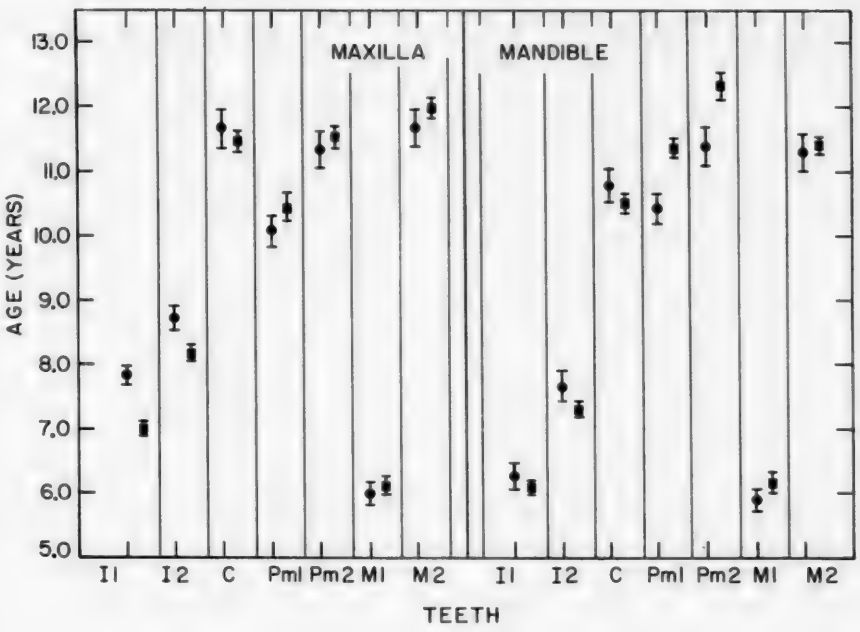
of information from each child. These medians, therefore, are not independent of each other. The formal method of choice for testing the significance of the difference between eruption times is a multivariate analysis of variance[10] by which a comparison is made of the total dentitions of the boys and girls. For our purposes, an informal comparison of the pattern shown is altogether convincing. An examination of the above tables and Fig. 2 on laterality, jaw, and sex differences among the Pima reveals the trends clearly.

Similarly, in comparing median age of tooth emergence between populations, the series of "t" tests is not suitable. Again the method of choice would be a multivariate analysis of variance. An informal comparison would be based on an inspection of the patterns in the critical ratios of the differences between the racial groups whose data are comparably analyzed. No significance levels can be given for the critical ratios because of the correlated nature of the data.

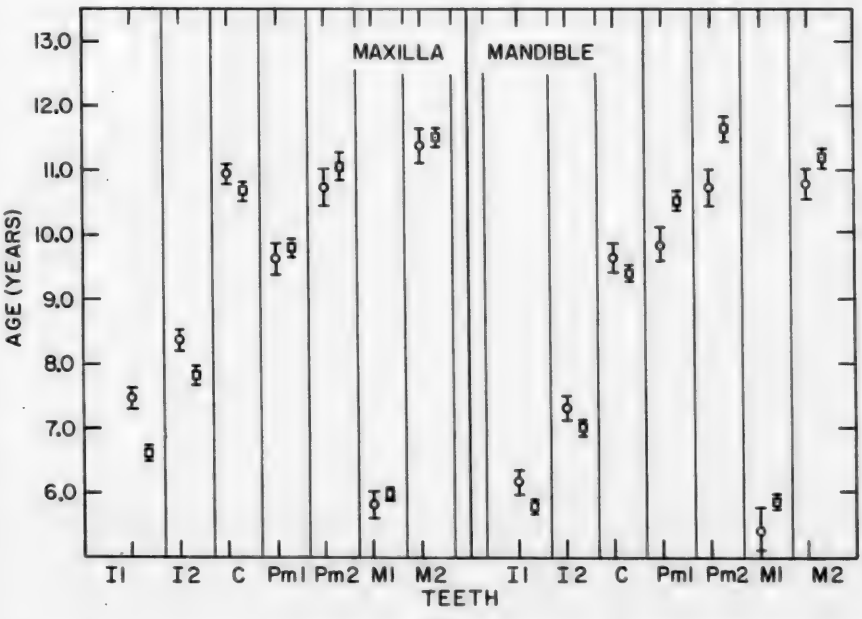
Comparability of methods of collecting data and of analyzing it is the primary consideration in the choice of studies to compare with the Pima Indian results. The studies chosen must be cross-sectional in design as is the Pima study. The tendency for longitudinal studies to give consistently later medians (Table I) is strong enough to warrant attention to this source of error. Of sets of data collected cross-sectionally, only those should be compared which have been analyzed by similar methods. However, as indicated above, this does not preclude some value from informal comparisons between miscellaneous groups. In the case of the Pima Indian data, the only suitable study is that of Clements and associates[2, 3] on English children in the Birmingham survey. It is cross-sectional data fitted to a regression line by the method of probits.

Table IX lists and Fig. 3 depicts the relationship between the Pima Indian population and the English children. The stability of a pattern similar in maxilla and mandible in both boys and girls is apparent. The anterior teeth of the Pima erupt later than do those of the English and the posterior teeth erupt earlier. Possible explanations for this pattern of relationship between the Pima Indian and English ages of dental eruption are: (a) the differences are genetically based and therefore are racial differences in the correct sense of the word, (b) they are environmentally induced, or (c) they are due to some complex of environmental and hereditary factors.

Leslie[16] discovered the same kind of a relationship between his New Zealand subjects and the English children studied by Ainsworth.[17] The anterior teeth of the New Zealand children erupted earlier and the posterior teeth later than those of the English children. One usual explanation for such a pattern is that the posterior teeth erupt earlier in a population subject to premature shedding of the deciduous molars. Leslie undertook to determine the effect of the age of shedding of the deciduous molars upon the eruption times of the succeeding posterior and adjacent posterior teeth.



A.



B.

Fig. 3.—A comparison of median ages of tooth emergence, Pima Indian versus English children, ± 2 standard errors of the median. *A*, Boys: ● = Pima, ■ = English. *B*, Girls: ○ = Pima, □ = English.

TABLE IX
A COMPARISON OF PIMA VERSUS ENGLISH MEDIAN AGES (IN YEARS) OF TOOTH EMERGENCE, THE STANDARD ERROR OF THE MEDIAN, AND CRITICAL RATIO OF DIFFERENCES

GROUP	MAXILLARY TEETH							MANDIBULAR TEETH						
	$^1I^1$	$^2I^2$	$^1C^1$	$^1Pm^1$	$^2Pm^2$	$^1M^1$	$^2M^2$	$_1I_1$	$_2I_2$	$_1C_1$	$_1Pm_1$	$_2Pm_2$	$_1M_1$	$_2M_2$
Boys														
Pima	7.83	8.74	11.66	10.08	11.33	5.98	11.67	6.26	7.65	10.78	10.43	11.39	5.89	11.29
S.E.	.07	.09	.14	.12	.14	.09	.14	.10	.12	.13	.12	.15	.09	.14
English	7.01	8.18	11.46	10.41	11.52	6.11	11.97	6.08	7.30	10.51	11.35	12.32	6.14	11.41
S.E.	.06	.06	.08	.09	.09	.07	.08	.06	.06	.07	.08	.10	.07	.07
Diff. P – E	.82	.56	.20	–.33	–.19	–.13	–.30	.18	.35	.27	–.92	–.93	–.25	–.12
C.R.	8.90	5.18	1.24	2.20	1.14	1.14	1.86	1.54	2.61	1.82	6.38	5.17	2.19	.08
Girls														
Pima	7.47	8.34	10.94	9.63	10.73	5.80	11.38	6.15	7.32	9.66	9.87	10.73	5.43	10.80
S.E.	.08	.08	.16	.12	.14	.10	.13	.09	.09	.11	.13	.14	.16	.12
English	6.62	7.82	10.67	9.79	11.06	5.94	11.50	5.77	7.01	9.41	10.53	11.64	5.84	11.18
S.E.	.06	.07	.07	.07	.10	.06	.07	.05	.06	.06	.08	.10	.06	.07
Diff. P – E	.85	.52	.27	–.16	–.33	–.14	–.12	.38	.31	.25	–.77	–.91	–.41	–.38
C.R.	8.50	4.90	2.29	1.15	1.92	1.20	.08	3.69	2.86	2.00	4.32	5.29	2.40	2.73

Effect of Deciduous Tooth Extraction.—From an examination of 248 girls and 255 boys between the ages of 10 and 11 years on whom complete dental histories were available for study, Leslie found the effect of deciduous molar extractions was to increase the total number of erupted premolars in the no-extractions experience group. Stones and co-workers[14] published data supporting this conclusion. The individual effects Leslie observed, however, were not so simple; they varied according to the age at which extraction occurred. The later the loss of the deciduous molars, the more accelerated the eruption of the succeeding premolars. The effects on the permanent molars could conceivably be similar to the effects on the premolars suggested by the results of Leslie and Stones. This is not an untenable position if one accepts as reasonable Leslie's explanations of the physiologic basis for accelerated eruption of premolars following on loss of the deciduous molars. He proposes that the removal of a deciduous tooth at a time when the resorption processes have been well established in the connective tissue adjacent to the erupting permanent tooth may enhance the rate of eruption of that tooth.

In the case of the Pima Indian children, an exceptional degree of wear on the deciduous molars to an extent rarely observed in white children is the rule. Excessive functional stresses on the deciduous molars are transmitted to the surrounding bone possibly causing accelerated resorption of the alveolar bone and subsequent advanced eruption of the premolars. This is especially noticeable in our data on the mandibular premolars of both boys and girls. Unfortunately, we have no exact figures on the dental health practices among the Pima which would be comparable to those of Leslie for the English and New Zealand children. However, the number of extractions because of caries and other reasons among the Pima children is very low. The DMF rates for the Pima by 1958 figures range roughly at the 2.55 level in the 6- to 11-year age group and lower for the older children. It is possible that the extreme wear on the deciduous molars of the Pima Indian children could cause earlier shedding through enhanced bone resorption under excessive functional stress.

The anterior dentition may reflect more directly, since deciduous incisor extractions are rare, the relationship between the two groups on fundamental rates of physical development. Leslie's data on height and weight of English and New England children support this idea. The advanced eruption of the anterior dentition of the New Zealand children is correlated with their superior height and weight. In the present instance, the advanced eruption of the anterior dentition of the English school children over the Pima children would imply commensurately advanced physical status. Considering the difference in diet and standard of living between Pima and English children, it is not difficult to imagine that part of the basis for earlier development among the English could be environmental.

On the hereditary aspects of tooth emergence, there is evidence in the literature[19, 20, 21] as well as in the Zoller Laboratory collection. Tisserand-Perrier's twin data are only one of several which are strongly suggestive that tooth emergence timing and sequence have a large hereditary component. Our family pedigree analyses indicate a high sibling correlation in age of dental eruption. The hereditary mechanisms operating in these families are, theoretically, capable of influencing an entire racial group, especially one as relatively small and inbred as is the Pima Indian population.

SUMMARY

The relative merits of using the normal probability function as the basis for the analysis of tooth emergence data are discussed and arguments advanced to support its use. The advantages of cross-sectional versus longitudinal studies of dental eruption are outlined.

Estimates of the population median ages of emergence of the permanent teeth of Pima Indian children are reported and compared to those of English children in the Birmingham survey. Possible explanations for the pattern of relations between the Indian and English children are discussed. Some evidence is presented for a complex of environmental and hereditary factors responsible for the observed relatively late eruption of the Pima anterior permanent teeth and relatively early eruption of the Pima posterior teeth when compared with the English children. Lower permanent second molars precede the emergence of the lower second premolars in the majority of instances among the Pima males.

REFERENCES

1. Garn, S. M., Koski, K., and Lewis, A. B.: Problems in Determining the Tooth Eruption Sequence in Fossil and Modern Man, *Am. J. Phys. Anthropol. 15 N.S.*, No. 3: 313, 1957.
2. Clements, E. M. B., Davies-Thomas, E., and Pickett, K. G.: Time of Eruption of Permanent Teeth in British Children in 1947-48, *Brit. M. J.* 1: 1421, 1953.
3. *Idem:* Order of Eruption of the Permanent Human Dentition, *Brit. M. J.* 1: 1425, 1953.
4. Klein, H., Palmer, C. E., and Kramer, M.: Studies in Dental Caries. II. The Use of the Normal Curve for Expressing the Age Distribution of Eruption of the Permanent Teeth, *Growth* 1: 386, 1938.
5. Finney, D. J.: Probit Analysis, Cambridge, 1952, Cambridge University Press.
6. Hurme, V. O.: Standards of Variation in the Eruption of the First Six Permanent Teeth, *Child Development* 19: 213, 1948. Reprinted *in* Basic Readings on the Identification of Human Skeletons. Estimation of Age. Editor-Trotter, 1954, Viking Fund, p. 35.

7. *Idem:* Ranges of Normalcy in the Eruption of Permanent Teeth, *J. Den. Children* Second Quarter, 1949.
8. Hurme, V. O., and Van Wagenen, G.: Basic Data on the Emergence of Deciduous Teeth in the Monkey (Macaca Mulatta), *Proc. Am. Phil. Soc.* **97**: 297, 1953.
9. Broca, P.: Instructions relatives à l'étude anthropologique du systeme dentaire, *Bull. Soc. Anthrop. de Paris* **2**: 128, 1879.
10. Rao, R.: Advanced Statistical Methods in Biometric Research, New York City, 1952, John Wiley & Sons, Inc.
11. Cattell, P.: Dentition as a Measure of Maturity, *Harvard Monographs in Education* No. 9, Boston, 1928, Harvard University Press.
12. Fulton, J. T., and Price, B.: Longitudinal Data on Eruption and Attack of the Permanent Teeth, *J. D. Res.* **33**: 65, 1954.
13. Lo, R. T., and Moyers, R. E.: Studies in the Etiology and Prevention of Malocclusion. I. The Sequence of Eruption of the Permaneat Dentition, *Am. J. Orthodont.* **39**: 460, 1953.
14. Stones, H. H., Lawton, F. E., Bransby, E. R., and Hartley, H. O.: Time of Eruption of Permanent Teeth and Time of Shedding of Deciduous Teeth, *Brit. D. J.* **90**: 1, 1951.
15. Hellman, M.: The Phase of Development Concerned With Developing the Permanent Teeth, *Am. J. Orthodont.* **29**: 507, 1943.
16. Leslie, G. H.: A Biometrical Study of the Eruption of the Permanent Dentition of New Zealand Children, Wellington, New Zealand, 1951, Gov't Printer.
17. Ainsworth, N. J.: Dental Disease in Children, *Med. Research Council, Great Britain,* Special Report, Series No. 97, 1925.
18. Steggerda, M., and Hill, T. J.: Eruption Time of Teeth Among Whites, Negroes, and Indians, *Am. J. Orthodont.* **28**: 361, 1942.
19. Suk, V.: Eruption and Decay of Permanent Teeth in Whites and Negroes, *Am. J. Phys. Anthropol.* **2**: 351, 1919.
20. Cohen, J. T.: The Dates of Eruption of the Permanent Teeth in a Group of Minneapolis Children, *J. A. D. A.* **15**: 2337, 1928.
21. Dahlberg, G., and Maunsbach, A. B.: The Eruption of the Permanent Teeth in the Normal Population of Sweden, *Acta Genetica* **1**: 77-91, 1948.

COBALT-INDUCED POLYCYTHEMIA

I. Clinical and Hematologic Observations

CLARK N. EID AND ROBERT J. GORLIN

Division of Oral Pathology, School of Dentistry, University of Minnesota, Minneapolis, Minn.

POLYCYTHEMIA has been grouped by Robbins[19] as follows:

1. *Relative polycythemia*—Caused by fluid loss from the body, such as is seen in burns, chronic diarrhea, or chronic vomiting.

2. *Secondary polycythemia*—Produced primarily by an anoxic condition in the red cell producing areas, specific examples being fetal marrow anoxia, poor circulation, high altitude, and chronic carbon monoxide poisoning.

3. *Polycythemia vera* (Vaquez-Osler disease)—Seen most commonly in adults from middle to later life. Relatively little is known about the etiology of this disease but some authors believe it to be a form of neoplasia.

Artificially produced polycythemia by the use of cobaltous salts seems to fit into the second group of polycythemias. The use of soluble cobalt salts in the induction of experimental polycythemia was first accomplished by Waltner and Waltner[24] upon the rat in 1929. Other investigators have confirmed their work using the rat,[1, 15, 17, 21, 24] dog,[4, 14] guinea pig,[22] mouse,[22] and rabbit.[9, 12] The means by which cobalt exerts its influence is not known. Levey and Orten[13] reported that polycythemia was not due to the formation of vitamin B_{12} as the content of this vitamin in the liver was not altered appreciably during administration of soluble cobalt salts. Many investigators seem to indicate that cobalt interferes with the sulfhydryl groups of enzymes which are active in cellular respiration,[3, 13, 16, 18] i.e., the enzymatic processes that are necessary for the utilization of oxygen at cellular levels. The relative anoxia would in turn stimulate the marrow to become hyperplastic.

Polycythemia may also be produced by atmospheric anoxia,[5, 20, 23] vasoconstrictors such as epinephrine, benzedrine,[6] and phenylephrine hydrochloride, and a pituitary erythropoietic factor.[13] The experimental polycythemia will not appear in hypophysectomized animals as a result of anoxia and/or the administration of cobalt salts.[13] It is possible that cobalt interferes with pituitary function with the resultant outpouring of the erythropoietic factor, thus stimulating erythropoiesis.

PILOT STUDY—MATERIALS AND METHODS

A pilot study was designed employing 18 adult male mice randomly divided into 6 control and 12 experimental animals. Cobaltous nitrate in a

This study was supported by a grant from the National Institutes of Health, United States Public Health Service.

Presented before the 36th Annual Meeting, I.A.D.R., Detroit, Mich., March 20-23, 1958.

Received for publication May 17, 1958; revised by authors July 7, 1958.

0.4 per cent solution was administered intraperitoneally at a level of 0.1 ml. per mouse per day. The weight of the mice averaging 30 grams, the dose was calculated to be 13.3 mg./Kg., i.e., 3.3 mg./Kg. more than the level used by Hopps, Stanley, and Shideler.[10] The control group received an equivalent amount of sterile physiologic saline. Red blood cell counts and hemoglobin values were obtained.

After 15 weeks the mice were sacrificed and histologic study of the kidney, liver, pancreas, spleen, adrenal gland, costochondral junction, temporomandibular joint, and jaws was undertaken.

Results of Pilot Study.—A maximal value of 17.02 million red blood cells per cubic millimeter was reached in one experimental mouse after 7 weeks of drug therapy; the lowest of the experimental group was 11.91 million while the highest control value was 11.89 million (Table I). No overlapping of the two groups at that one week was observed. At the fifteenth week, the lowest experimental value was 3.21 million red blood cells per cubic millimeter and the lowest control was 10.09 million. The highest experimental value on that date was 14.68 million. Anemia was seen in 2 animals and a third animal showed a borderline condition (6 million red blood cells per cubic millimeter). These initial studies demonstrated that the red blood cell counts increased and then subsequently decreased in the experimental animals.

TABLE I
MEAN RED BLOOD CELL COUNTS—MICE

GROUP	INITIAL	7 WEEKS	13 WEEKS	15 WEEKS
Control	9,170,000	9,700,000	10,080,000	10,760,000
Experimental	9,130,000	14,420,000	10,990,000	10,300,000
Net change	−40,000	+4,720,000	+910,000	−460,000

No significant histopathologic changes were noted in any of the organs or bony structures of the experimental animals, verifying the observations of Hopps and associates.[10]

The mouse appeared to be a rather unsatisfactory animal for hematological studies, primarily, because of its small total blood volume and the relative difficulty in obtaining enough blood for blood counts and hemoglobin determination without appreciably affecting the results. As 2 experimental animals apparently died of anemia, it was of interest to know whether the anemia was induced by the cobalt ion or by the investigative laboratory methods employed.

METHODS

After consultation with the Biostatistics Division of the University of Minnesota, an experiment was designed using 50 albino rats, 42 days of age, equally divided according to sex, randomly distributed, and individually marked by ear punching. Thus, there were 12 male controls, 13 male experimentals, 12 female controls, and 13 female experimental animals. Each of the 4 groups was individually housed with sufficient water and food (Purina Fox Chow). Carrots and potatoes were given weekly to all groups as a supplement to their diet.

Weekly body weights, red blood cell counts, hemoglobin values, and differential white cell counts were taken. The animals were injected daily on the basis of each individual rat's weight. Experimental animals received 0.1 ml. of 0.4 per cent cobaltous nitrate intraperitoneally per 40 grams of body weight per day. This dose was calculated to be 10.0 mg. per kilogram (3.3 mg. per kilograms less than the dose of the pilot study). After 3 weeks elapsed, the experimental animals manifested a paralysis of the hindquarters for about

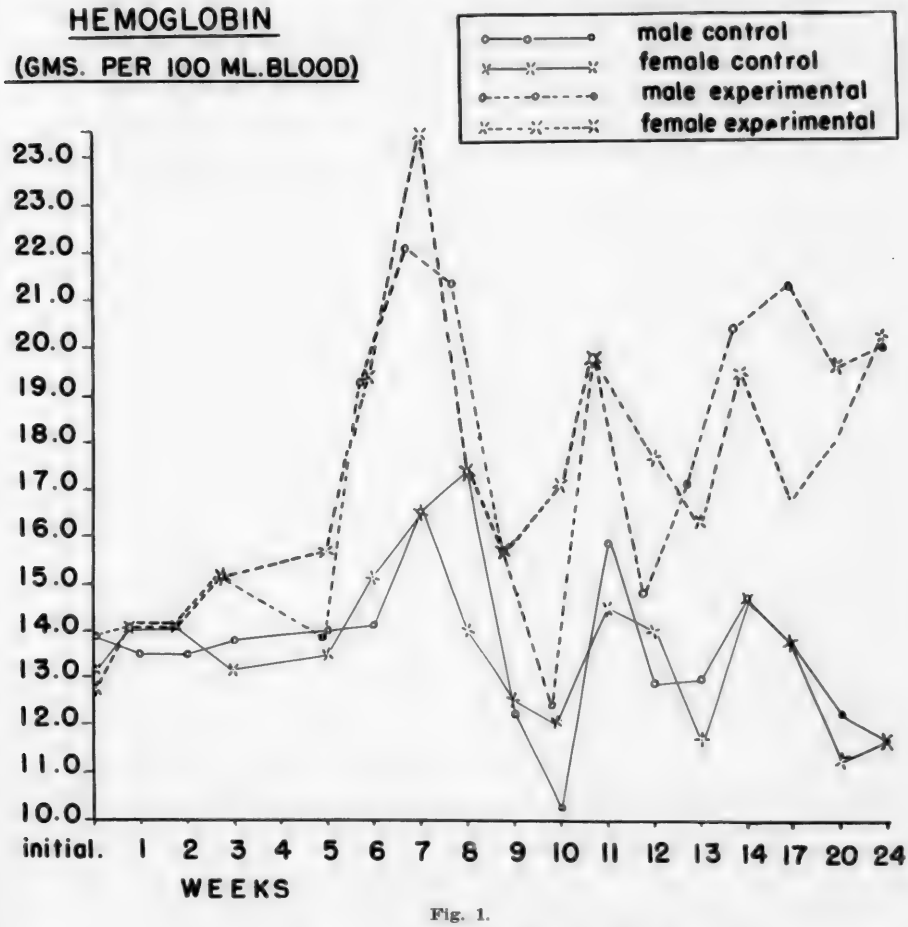


Fig. 1.

one-half hour following the injection. Accordingly, the dose was reduced to 0.1 ml. per 50 grams of body weight, i.e., 8 mg. per kilogram of body weight. The control dose of isotonic saline was varied accordingly, i.e., from 0.1 ml. per 40 grams to 0.1 ml. per 50 grams of body weight.

The red blood cell counts, hemoglobin values, differential white cell counts and weights were recorded prior to the first injections and weekly thereafter for 14 weeks, then at the seventh, twentieth, and twenty-fourth weeks.

The blood picture of rat: Newborn rats have 75 per cent of the adult hemoglobin value of 15.6 grams per 100 ml. of blood and only 2.5 to 3.5 million red blood cells per cubic millimeter. Four months are required for adult values to be attained.[8]

RESULTS

About 4 to 5 weeks were required before any appreciable difference between the control and experimental animals was noticed.

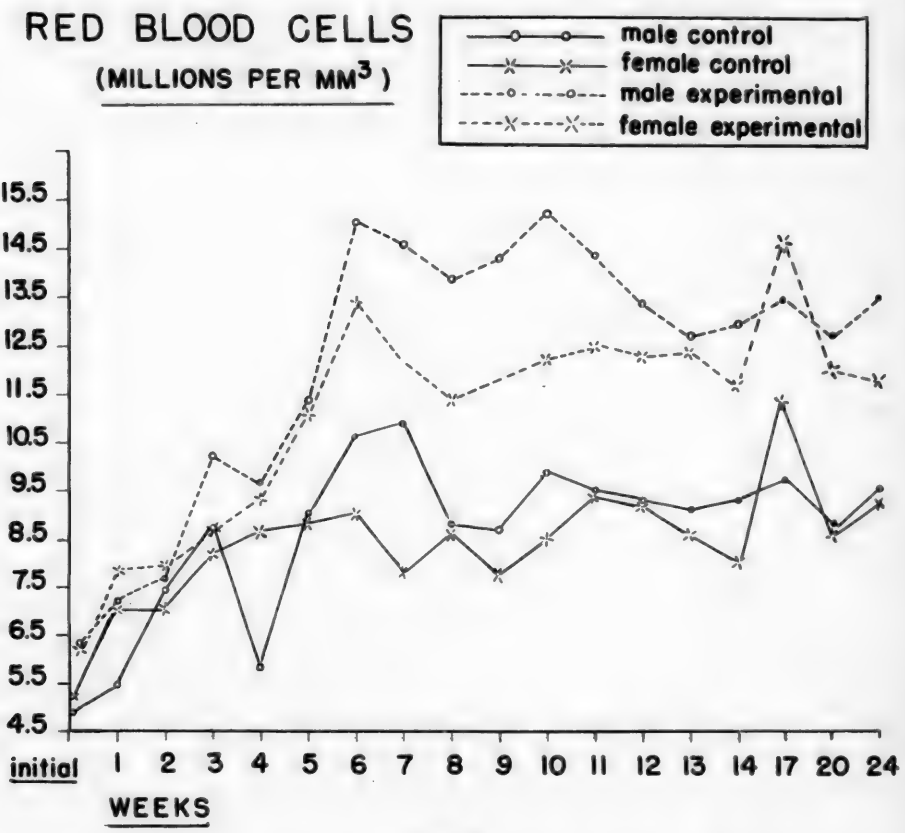


Fig. 2.

At 7 to 8 weeks the mean hemoglobin values reached their maximum levels for all groups. These values compared very favorably to those of Levey and Orten.[13] At the end of 8 weeks their controls averaged 14.7 grams per 100 ml. blood and their experimental group averaged 10.4 grams (Fig. 1).

The mean red cell counts reached their maximum levels anywhere from 7 to 11 weeks; the female experimentals reached these levels again at 17 weeks. This did not mean that the entire group hit their maximum levels at that time, for the experimental groups required from 6 to 10 weeks for any individual

animal to reach its peak. It is interesting to note that the time required for the hemoglobin maximum to be reached was 7 weeks while the red blood cell count maximum was not attained until 9 to 10 weeks in the experimental groups (Fig. 2).

Mean corpuscular hemoglobin values were quite similar between the experimental and control groups for both males and females (Fig. 3).

In the female experimental group of 6 to 7 weeks, the mean red blood cell count dropped from 13.49 to 12.29 million while the hemoglobin values went from 19.4 to 24.2 grams per 100 ml. of blood. This at first appeared to be a supersaturation but the mean corpuscular hemoglobin correspondingly went from 0.144×10^{-7} to 0.197^{-7} mg. per cell, a value less than that initially observed. It

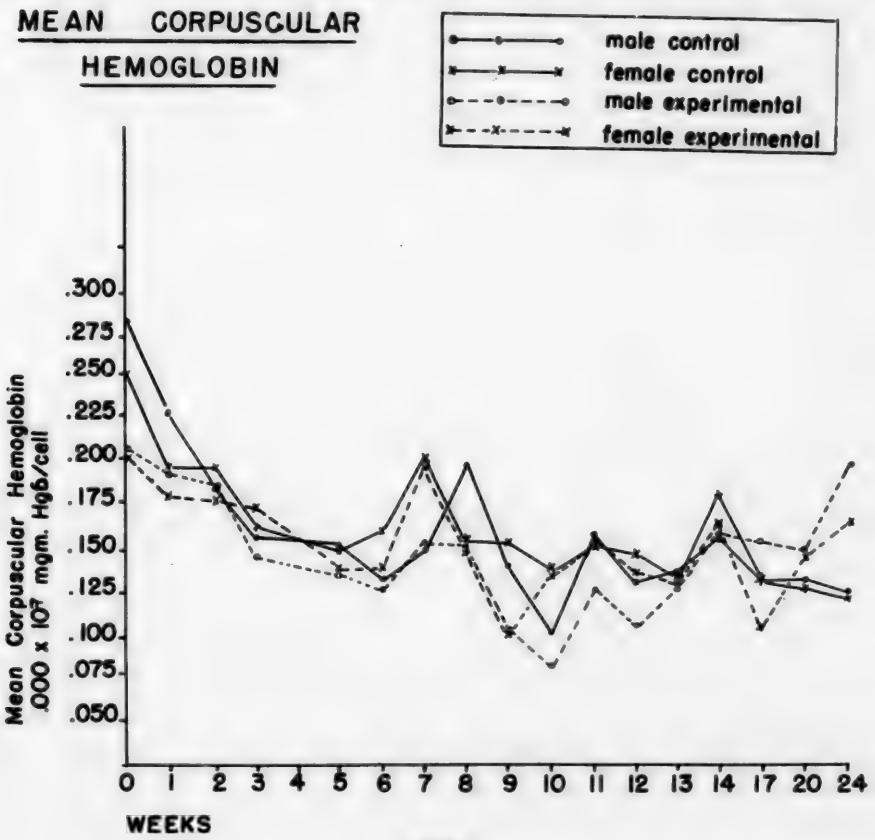


Fig. 3.

was also noted that the mean corpuscular hemoglobin of the experimental animals was less than that of the respective controls except in 9 cases of the 36 corresponding groups (i.e., in any one week two groups were run—male and female). This seems significant for it suggests that the red blood cell in this secondary polycythemia did not have an oxygen carrying capacity equivalent to

that of the red blood cell of the normal animal until 17 weeks had elapsed; then the picture was reversed. However, because of the over-all increase in the red blood cell count in the experimental groups, both before and after the preceding transition had taken place, the experimental animal's total oxygen carrying capacity was considerably above that of the control animal.

There was also a remarkable weight difference. In general, males are larger than females. At the end of 24 weeks the male controls averaged 440 grams while the male experimental average was 300 grams, a difference of 140 grams.

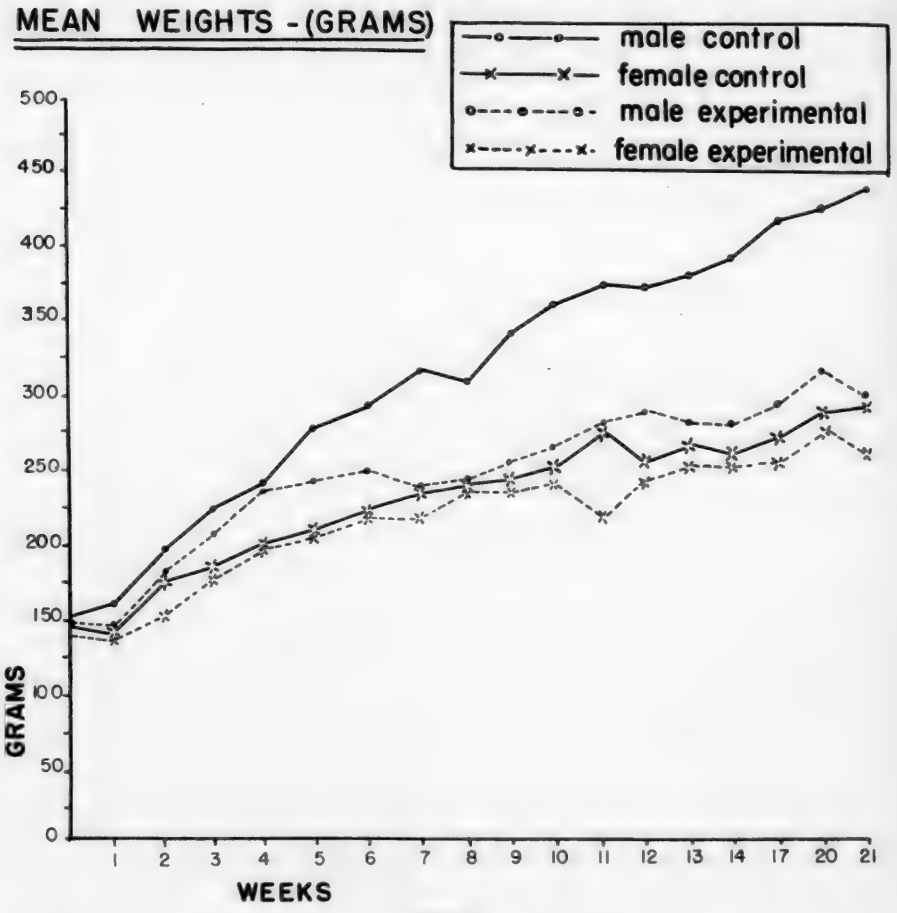


Fig. 4.

The female control average weight at the end of 24 weeks was 291 grams while the mean female experimental weight was 263 grams. The difference of 28 grams was not statistically significant (Fig. 4).

Differential white cell studies were not significantly different. The lymphocyte counts of the experimental animals were a little lower than those of the

control animals but not significantly so. The decrease in the lymphocyte count was made up by an increase in the polymorphonuclear neutrophils. The eosinophil counts indicated that control animals have a few more eosinophils. Since diarrhea was noted during the thirteenth and fourteenth weeks and the eosinophil and neutrophil counts were considerably higher than normal in all groups, it was decided to defer blood studies for 3 weeks to allow the animals to return to normal—which they did. The individual weights and injections were, however, continued as usual.

Microhematocrits were performed only in the twenty-fourth week. The female control mean was 46.9 per cent, the female experimental mean was 70.1; the male control mean was 45.3 per cent and the male experimental mean was 74.3 per cent. The control hematocrit values compared very favorably to 49 per cent for males and 52 per cent for females reported by Farris and Griffith.[8]

Histologic studies have been undertaken of the marrow, teeth, cortical and cancellous bone, costochondral junction, liver, spleen, kidney, and adrenal glands. This work will be reported at a later date.

DISCUSSION

A large weekly variation was observed in the mean corpuscular hemoglobin due to mean hemoglobin values undergoing marked variation from one week to the next. This was especially true in the experimental group. Since neither a change of investigators nor a change in technic had been effected, this hemoglobin variation was impossible to explain. Perhaps the explanation lies in the color comparison method used for hemoglobin determination which does have considerable inherent error.

Perhaps the most unusual observation was that of growth suppression of the male experimental group. Statistical evaluation of the data revealed that the weight difference was highly significant. Whether this reflects the effect of the cobalt ion upon the pituitary gland cannot be determined from this experiment.

SUMMARY

1. Polycythemia was produced in mice and rats after injection of a cobaltous salt for 4 to 5 weeks. Red blood cell counts leveled off by the eighth week and remained more or less constant.

2. Hemoglobin values were quite regular for the controls and very irregular for the experimental animals.

3. The mean corpuscular hemoglobin is less in the experimental animals than in the controls for 14 weeks.

4. Maximum hemoglobin values were reached about 2 weeks earlier than maximum red blood cell counts.

5. No difference was noted in the differential white blood cell counts between the control and experimental groups.

6. Cobaltous nitrate seems to slow the rate of growth in male rats but not in females.

7. The effective dose on a weight basis is different for rats and for mice. An intraperitoneal overdose of cobaltous nitrate in rats is manifest by a temporary paralysis of the hindquarters.

8. Histologic study of the organs and bones of adult male mice with 15 weeks of cobalt administration has shown no significant changes.

REFERENCES

1. Brand E., and Stucky, C. J.: Cobalt Glutamate in Nutritional Anemia, *Proc. Soc. Exper. Biol. & Med.* **31**: 739, 1934.
2. Bruner, H. D., van de Erve, J., and Carlson, A. J.: The Blood Picture of Rats from Birth to Twenty-Four Days of Age, *Am. J. Physiol.* **124**: 620, 1938.
3. Burk, D., Caroline, L., and Schade, A. L.: Reversible Complexes of Cobalt, Histadine, and Oxygen Gas, *J. Biol. Chem.* **165**: 723, 1946.
4. Davis, J. E.: Cobalt Polycythemia in the Dog, *Proc. Soc. Exper. Biol. & Med.* **37**: 96, 1937.
5. Davis, J. E.: The Mechanism of the Depressant Action of Liver and Choline Hydrochloride Upon Experimental Polycythemia. The Effect of Sodium Nitrate and Choline Derivatives on Polycythemic Dogs, *J. Pharmacol. & Exper. Therap.* **70**: 408, 1940.
6. Davis, J. E.: The Production of Experimental Polycythemia by the Daily Administration of Epinephrine or Posterior Pituitary Solution, *Am. J. Physiol.* **137**: 699, 1942.
7. Dorland, W. A.: The American Illustrated Medical Dictionary, ed. 22, Philadelphia and London, 1951, W. B. Saunders Company.
8. Farris, E. J., and Griffith, J. Q.: The Rat in Laboratory Investigation, ed. 2, Philadelphia, London, Montreal, 1947, J. B. Lippincott Company, pp. 81, 407, 410.
9. Goodman, J.: Production of Polycythemia in Rabbits by Anoxia and Cobalt, *Proc. Soc. Exper. Biol. & Med.* **64**: 336,1947.
10. Hopps, H. C., Stanley, A. J., and Shideler, A. M.: Polycythemia Induced by Cobalt: III. Histologic Studies With Evaluation of Toxicity of Cobaltous Chloride, *Am. J. Clin. Path.* **24**: 1374, 1954.
11. Hickling, R. A.: Gout, Leukemia and Polycythemia, *Lancet* **1**: 57, 1953.
12. Kleinberg, W.: The Hemopoietic Effect of Cobalt and Cobalt Manganese Compounds in Rabbits, *Am. J. Physiol.* **108**: 545, 1934.
13. Levey, S., and Orten, J. M.: Vitamin B_{12} and Production of Polycythemia by Cobalt, *J. Nutrition* **45**: 487, 1951.
14. Mascherpa, P.: Le pouvoir hematopoietique de cobalt, *Arch. ital. de biol.* **82**: 112, 1930.
15. Meyers, V. C., Beard, H. H., and Barnes, B. O.: Studies in the Nutritional Anemia of the Rat: IV. The Production of Hemoglobinemia and Polycythemia in Normal Animals by Means of Inorganic Elements, *J. Biol. Chem.* **94**: 117, 1931.
16. Michaelis, L.: The Magnetic Properties of the Cobalt-Histadine Complex Before and After Oxygenation, *Arch. Biochem.* **14**: 17, 1947.
17. Orten, J. M., Underhill, F. A., Mugrage, E. R., and Lewis, R. C.: Production of Experimental Polycythemia With Cobalt, *Proc. Soc. Exper. Biol. & Med.* **29**: 174, 1931.
18. Orten, J. M., and Bucciers, M. C.: The Effect of Cysteine, Histadine and Methionine on the Production of Polycythemia by Cobalt, *J. Biol. Chem.* **176**: 961, 1948.
19. Robbins, S. L.: Textbook of Pathology, Philadelphia and London, 1957, W. B. Saunders Company, p. 568.
20. Smith, W. W., Cool, W. S., Smith, F., and Altland, P. D.: Effect of Altitude—Induced Polycythemia and Reticulocytosis on Tolerance of Rats to Radiation, *Am. J. Physiol.* **170**: 396, 1952.
21. Stanley, A. J., Hopps, H. C., and Hellbourn, A. A.: Observation on Cobalt Polycythemia. I. Studies on the Peripheral Blood of Rats, *Proc. Soc. Exper. Biol. & Med.* **61**: 130, 1946.
22. Sutter, J.: Cobalt et hyperglobulie, *Comptes rend. Soc. de biol.* **116**: 994, 1934.
23. Thorn, G. W., Jones, B. F., Lewis, R. A., Mitchell, E. R., and Heopf, G. F.: The Role of the Adrenal Cortex in Anoxia; The Effect of Repeated Daily Exposures to Reduced Oxygen Pressure, *Am. J. Physiol.* **137**: 606, 1942.
24. Waltner, K., and Waltner, K.: Kobalt und blut, *Klin. Wchnschr.* **8**: 313, 1929.

EFFECT OF DELETION OF BIVALENT TRACE MINERALS ON GROWTH AND GLYCOLYTIC ACTIVITY OF ORAL ACIDOGENIC MICROORGANISMS*

SAMUEL DREIZEN, WILLIAM NIEDERMEIER, JO G. DREIZEN, AND TOM D. SPIES

The Department of Nutrition & Metabolism, Northwestern University, Chicago, Ill.

STUDIES of the trace elements required for the growth of oral acidogenic microorganisms and for glycolysis in saliva-glucose mixtures have been concerned primarily with the effect of added minerals on acid production.[1-3] The degree to which removal of naturally occurring trace elements in human saliva may affect carbohydrate degradation by the oral flora has not been determined. With the development and study of a great many chelating compounds, selective deletion of specific trace minerals from biologic material can now be accomplished to a considerable extent.

It is generally accepted that chelating agents act by forming coordination complexes with metallic ions and that metals bound in this manner are physiologically and biochemically inactive.[4] Addition of soluble salts of the bound minerals in quantities which exceed the combining capacity of a chelator overcomes the inactivating effect either partially or completely. Selected chelating compounds have been employed in the present series of investigations to elicit the bivalent metallic ions required for the growth and acid production of oral acidogens in saliva-glucose mixtures and in synthetic and chemically defined bacteriologic media. For ease of presentation, details of these studies are described separately.

STUDY NO. 1. EFFECT OF INACTIVATION OF BIVALENT METALS ON ACID PRODUCTION IN SALIVA-GLUCOSE MIXTURES

Materials and Methods.—Five organic chelating compounds were selected from the very large group of commercially available agents compiled by Albert and Gledhill.[5] These consisted of oxine (8-hydroxyquinoline) with a weak affinity for Fe^{++} and a strong attraction for Mn^{++}, Zn^{++}, Cd^{++}, Co^{++}, Pb^{++}, and Cu^{++}; sodium diethyldithiocarbamate with a weak capacity to bind Mn^{++} and Fe^{++} and a marked affinity for Zn^{++}, Cd^{++}, Co^{++}, Pb^{++}, and Cu^{++}; nitroso-R-salt (sodium 1-nitroso-2-naphthol-3,6-disulphonate) with a pronounced attraction for Fe^{++}, Co^{++}, and Cu^{++}; and titan yellow (sodium 4,4′-di-(6-methyl-2-benzthiazolyl)-diazoaminobenzene-2,2′-disulphonate) and thiourea each with a weak affinity for Cu^{++}.

The antiglycolytic effect of the removal of the aforementioned minerals was determined by adding 0.25 ml. and/or 0.50 ml. of 0.02 M concentrations of the 5 chelators to separate sterile brown vials containing a 10 ml. aliquot of

These studies were supported by grants from the Charles F. Kettering Foundation, Inc., and the Avondale Educational and Charitable Foundation.

Received for publication May 22, 1958; revised by authors Sept. 29, 1958.

*Northwestern University studies in nutrition at the Hillman Hospital, Birmingham, Ala.

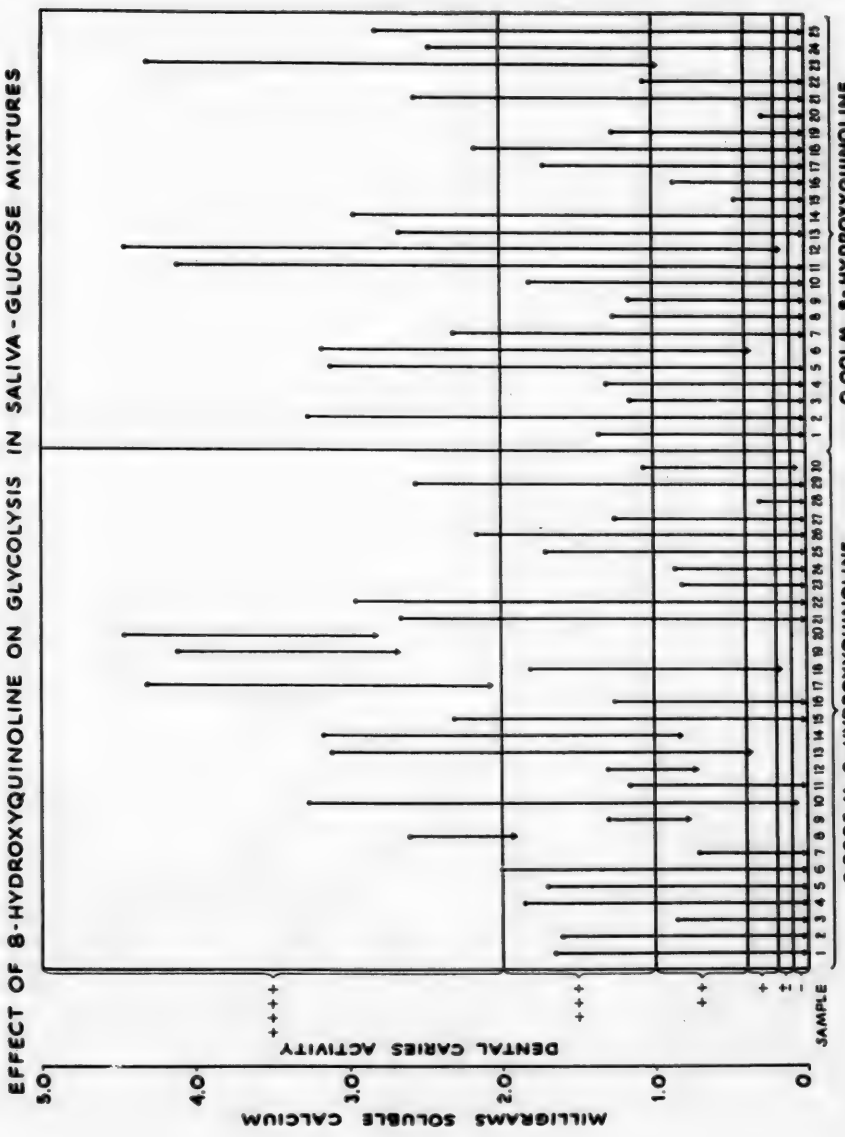

EFFECT OF 8-HYDROXYQUINOLINE ON GLYCOLYSIS IN SALIVA-GLUCOSE MIXTURES
MILLIGRAMS SOLUBLE CALCIUM
5.0
4.0
3.0
2.0
1.0
0
DENTAL CARIES ACTIVITY
++++
+++
++
+
±
-
SAMPLE
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
0.0005 M 8-HYDROXYQUINOLINE
0.001 M 8-HYDROXYQUINOLINE

Fig. 1.

paraffin-stimulated human saliva, 100 mg. glucose, and 100 mg. tricalcium phosphate. The final concentrations of the chelating agents in the saliva-glucose-tricalcium phosphate mixtures were 0.0005 M and 0.001 M, respectively. None of the 5 chelators binds Ca^{++} at these concentrations.[5] Acid production was measured indirectly by the method of Fosdick, Hansen, and Epple.[6] In this method, an untreated 10 ml. aliquot of a saliva sample is analyzed immediately after collection for soluble calcium. The aliquot containing chelator, glucose and tricalcium phosphate, is incubated and agitated in a water bath maintained at 37° C. for 4 hours at the end of which time the amount of soluble calcium present therein is determined. The difference in the soluble calcium content between the incubated and unincubated aliquots reflects the amount of acid formed by microbial fermentation of glucose.

The glycolytic inhibitory potential of oxine was tested in 55 samples of paraffin-stimulated whole saliva obtained from patients with clinical and laboratory evidence of dental caries activity. In 30 samples, oxine was added in quantities sufficient to make a 0.0005 M concentration of the chelator; in 25 cases the final oxine concentration was 0.001 M. Nitroso-R-salt, sodium diethyldithiocarbamate, titan yellow, and thiourea were each added to 36 samples of saliva, 18 at a chelator concentration of 0.0005 M and 18 at a concentration of 0.001 M.

Observations.—As shown in Figure 1, oxine completely inhibited acid production in 21 (70 per cent) of the 30 samples of saliva at a level of 0.0005 M and was partially inhibitory in the remaining 9 (30 per cent) cases. At a 0.001 M concentration of oxine, glycolysis in the saliva-glucose-tricalcium phosphate mixtures was completely prevented in 22 (88 per cent) and partially inhibited in 3 (12 per cent) of the 25 samples. Neither nitroso-R-salt (Fig. 2), thiourea, nor titan yellow materially affected glycolysis in the saliva-glucose samples to which they were added. A 0.001 M concentration of sodium diethyldithiocarbamate completely inhibited acid formation in 4 (22 per cent) samples, was partially inhibitory in 13 (73 per cent), and ineffective in 1 (6 per cent) (Fig. 2); at the 0.0005 M concentration, corresponding results were obtained in 3 (17 per cent), 12 (67 per cent), and 3 (17 per cent) of the samples, respectively.

STUDY NO. 2. EFFECT OF CHELATION OF BIVALENT METALS ON GROWTH OF ORAL ACIDOGENS IN SYNTHETIC AND CHEMICALLY DEFINED BACTERIOLOGIC MEDIA

Materials and Methods.—Mixed cultures of lactobacilli, streptococci, and staphylococci were isolated from 12 samples of saliva which showed an exceedingly high acid-producing capacity in the previous study. Each mixture was grown in thioglycollate-glucose broth for 24 hours at 37° C. The 24-hour cultures were then centrifuged, the supernatant discarded, and the organisms washed 3 times in physiologic saline.

One drop of each washed culture was seeded into 2 series of duplicate tubes. One series contained 10 ml. thioglycollate-glucose broth per tube; the other, 10 ml. of a modified chemically defined medium described by Bentley, Snell, and Phillips[7] for the microbiologic determination of manganese. The composition of this medium is shown in Table I. Manganese chloride was added to the

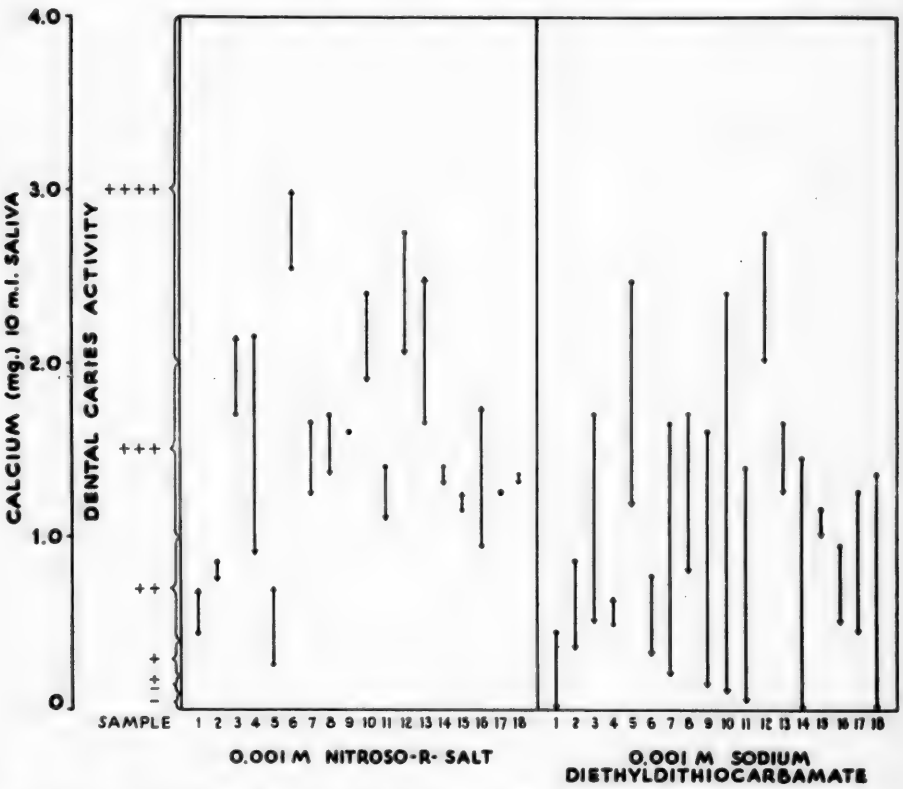


Fig. 2.—Effect of 0.001 M nitroso-r-salt and of 0.001 M sodium diethyldithiocarbamate on glycolysis in saliva-glucose mixtures.

TABLE I
COMPOSITION OF CHEMICALLY DEFINED TEST MEDIUM

CONSTITUENT	QUANTITY
Casein hydrolysate	20 ml.
Glucose	8 Gm.
Sodium acetate	4 Gm.
Salt solution A*	2 ml.
Salt solution B†	16 ml.
Cystine	160 mg.
Adenine sulfate	8 mg.
Guanine hydrochloride	8 mg.
Uracil	8 mg.
Tryptophane	80 mg.
Asparagine	200 mg.
Folic acid	100 μg
Biotin	100 μg
Riboflavin	160 μg
Calcium pantothenate	160 μg
Nicotinic acid	160 μg
Thiamine hydrochloride	80 μg
Pyridoxine hydrochloride	320 μg
Distilled water to 400 ml.	

Medium adjusted to pH 6.8 with NaOH.

*Salt solution A: KH_2PO_4, 20 Gm.; K_2HPO_4, 20 Gm.; distilled water to 200 ml.

†Salt solution B: $MgSO_4 \cdot 7\ H_2O$, 10.0 Gm.; NaCl, 0.5 Gm.; $FeSO_4 \cdot 7\ H_2O$, 0.5 Gm.; distilled water to 250 ml.

inoculated control tubes at a level of 0.0005 M $MnCl_2$ per tube. Each of the 5 chelators was added to the duplicate tubes in both series in amounts which produced 0.0005 M and 0.001 M concentrations of these agents. The tubes were incubated at 37° C. for 72 hours. Growth was determined turbidimetrically and acid production measured by titration with 0.1 N NaOH to the pH of uninoculated control tubes. A Beckman Model G pH meter was used for this purpose.

Observations.—Growth and acid production were completely inhibited in thioglycollate-glucose broth containing 0.0005 M oxine in 9 (75 per cent) of the mixed cultures; at 0.001 M oxine, complete inhibition was observed in 11 (92 per cent) cultures. In the remaining cultures, acid formation varied from 35.3 to 76.1 per cent of that in inoculated control tubes lacking the chelator (Table II). In the chemically defined medium, 10 (83 per cent) of the 12 mixed

TABLE II
EFFECT OF 0.0005 M AND 0.001 M 8-HYDROXYQUINOLINE (OXINE) ON ACID PRODUCTION BY MIXED CULTURES OF ORAL LACTOBACILLI, STREPTOCOCCI, AND STAPHYLOCOCCI IN THIOGLYCOLLATE-DEXTROSE BROTH*

	INOCULATED CONTROL		0.0005 M OXINE		0.001 M OXINE		0.0005 M OXINE + 0.0005 M $MnCl_2$		0.001 M OXINE + 0.0005 M $MnCl_2$	
CULTURE NUMBER	ML. 0.1 N NaOH	%	ML. 0.1 N NaOH	%	ML. 0.1 N NaOH	%	ML. 0.1 N NaOH	%	ML. 0.1 N NaOH	%
1	3.60	100	2.75	76.1	0.00	0.0	4.10	113.9	4.00	111.1
2	2.60	100	0.00	0.0	0.00	0.0	2.00	55.5	0.00	0.0
3	2.65	100	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
4	2.75	100	0.00	0.0	0.00	0.0	3.25	118.2	3.20	116.0
5	3.20	100	0.00	0.0	0.00	0.0	1.70	53.1	0.60	18.8
6	2.50	100	0.00	0.0	0.00	0.0	3.15	126.0	3.50	140.0
7	3.30	100	0.00	0.0	0.00	0.0	3.30	100.0	0.00	0.0
8	3.20	100	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
9	3.40	100	1.30	38.2	0.00	0.0	2.90	85.3	3.40	100.0
10	3.40	100	1.45	42.6	1.20	35.3	3.25	95.6	3.90	114.7
11	3.20	100	0.00	0.0	0.00	0.0	3.90	121.9	4.00	125.0
12	3.15	100	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0

*Each tube of 10 ml. thioglycollate-dextrose broth contained 0.26 μg Mn.

cultures failed to grow or produce acid in the presence of 0.0005 M oxine and 11 (92 per cent) were completely inhibited by 0.001 M oxine. In all other tubes containing oxine, acid production was partially prevented, the amounts formed ranging from 53.9 to 65.9 per cent of the inoculated control tubes (Table III). Growth and acid production by the 12 mixed cultures were essentially unaffected by 0.0005 M and 0.001 M concentrations of nitroso-R-salt, titan yellow, thiourea, and sodium diethyldithiocarbamate.

STUDY NO. 3. EFFECTIVENESS OF MANGANESE IN REVERSING THE INHIBITORY ACTION OF OXINE IN SALIVA-GLUCOSE MIXTURES AND IN BACTERIOLOGIC MEDIA

Materials and Methods.—The distinguishing feature of oxine in contrast to the other 4 chelators is its ability to chelate manganese. None of the other agents chelates manganese to an appreciable extent, although sodium diethyldithiocarbamate has a very weak affinity for this element. In view of the demonstrable deterring power of oxine on acid production in saliva-glucose mixtures

and on the growth of mixed oral acidogens, the preceding studies were extended by adding 0.50 ml. 0.01 M $MnCl_2$ to a third 10 ml. aliquot of the saliva-glucose-tricalcium phosphate mixtures used in Study No. 1 and to tubes containing 10 ml. thioglycollate-glucose broth and chemically defined test medium. Twenty-two samples of saliva with 0.0005 M oxine and 15 samples with 0.001 M oxine were examined in this manner. The growth promoting properties of added manganese in the presence of oxine were tested in the 12 mixed cultures employed in Study No. 2. The subsequent experimental procedures were identical with those described above.

TABLE III
EFFECT OF 0.0005 M AND 0.001 M 8-HYDROXYQUINOLINE (OXINE) ON ACID PRODUCTION BY MIXED CULTURES OF ORAL LACTOBACILLI, STREPTOCOCCI, AND STAPHYLOCOCCI IN THE CHEMICALLY DEFINED MEDIUM

	INOCULATED CONTROL + 0.0005 M $MnCl_2$		0.0005 M OXINE		0.001 M OXINE		0.0005 M OXINE + 0.0005 M $MnCl_2$		0.001 M OXINE + 0.0005 M $MnCl_2$	
CULTURE NUMBER	ML. 0.1 N NaOH	%	ML. 0.1 N NaOH	%	ML. 0.1 N NaOH	%	ML. 0.1 N NaOH	%	ML. 0.1 N NaOH	%
1	9.65	100	5.20	53.9	0.00	0.0	9.70	100.5	9.75	101.0
2	10.80	100	0.00	0.0	0.00	0.0	6.25	56.9	0.00	0.0
3	9.85	100	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
4	10.25	100	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
5	12.85	100	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
6	10.85	100	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
7	10.70	100	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
8	9.80	100	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
9	9.70	100	6.40	65.9	6.40	65.9	9.60	98.9	9.70	100.0
10	9.20	100	0.00	0.0	0.00	0.0	9.30	101.1	9.30	101.1
11	12.60	100	0.00	0.0	0.00	0.0	8.30	65.9	0.00	0.0
12	10.00	100	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0

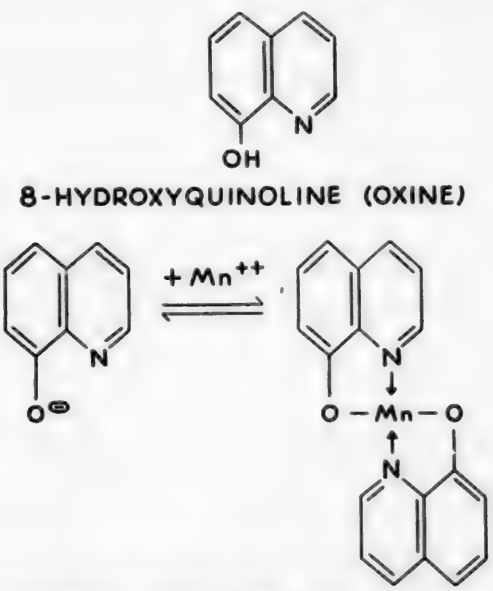
Observations.—The addition of $MnCl_2$ resulted in a partial to complete reversal of inhibition of acid production in 17 (77 per cent) of the 22 samples of saliva with 0.0005 M oxine and in 12 (80 per cent) of the 15 samples containing 0.001 M oxine. A similar effect was noted in 9 (75 per cent) of the 12 mixed cultures inoculated into thioglycollate-glucose broth containing 0.0005 M oxine and in 7 (58 per cent) of the cultures exposed to 0.001 M oxine (Table II). In the chemically defined medium, reversal of oxine inhibition occurred in 5 (41 per cent) of the mixed cultures subjected to 0.0005 M oxine and in 3 (25 per cent) containing 0.001 M oxine (Table III).

STUDY NO. 4. THE MANGANESE CONTENT OF HUMAN WHOLE SALIVA

Materials and Methods.—The manganese content of paraffin-stimulated human whole saliva was determined by the method of Gates and Ellis,[8] the only change being in the ashing procedure. Fifteen 100 ml. samples of stimulated saliva collected from patients and personnel of the Nutrition Clinic were evaporated to dryness and ashed for 4 hours at 550° C. After cooling, 2 ml. of 50 per cent redistilled nitric acid were added to each residue and returned to the muffle furnace for another 2 hours. This method yielded a white ash which

was readily soluble in a mixture of dilute nitric and phosphoric acids. The manganese content of the thioglycollate medium used in these studies was determined in a similar fashion on one gram samples.

Observations.—Each of the 15 samples of saliva contained a detectable quantity of manganese. The content ranged from 0.0011 to 0.017 μg per milliliter with a mean of 0.005 μg per milliliter. The thioglycollate medium contained 1.10 μg manganese per gram of dry weight or 0.026 μg per milliliter of broth.



REACTION BETWEEN TWO OXINE ANIONS AND ONE MANGANOUS ION TO GIVE OXINE-METAL CHELATE COMPLEX

Fig. 3.

DISCUSSION

In a symposium devoted to the lactic acid bacteria in 1952, Tittsler and associates[9] noted that because of the many organic compounds required by such bacteria and the lack of technics for freeing these of traces of inorganic ions, detailed knowledge of the requirements of these organisms for such ions is lacking. Previously, MacLeod and Snell,[10] employing microbiologic assay procedures, had shown that lactic acid bacteria require relatively large amounts of potassium, manganese, and phosphates, with magnesium being essential for some strains. Subsequently, Carlson, Farina, and Whiteside-Carlson[11] found that manganese is a nutritional essential for a strain of oral *Lactobacillus acidophilus*. Gale[12] demonstrated that manganese is needed for the assimilation of glutamic acid by some bacteria and that the addition of oxine inhibits this process by combining with manganese. The chelating action of oxine was shown

by Albert and associates[18] to result from its combining with metals to form five-membered rings in which the coordinate bond between metal and nitrogen consists of two electrons supplied by the latter (Fig. 3).

The present study utilizing oxine and 4 other chelating agents to inactivate bivalent metallic ions demonstrates the essentiality of manganese for acid production by oral acidogens both in saliva-glucose mixtures and in bacteriologic media. Conversely, chelation of traces of copper, cobalt, zinc, lead, cadmium, and iron which might have been present in saliva and in the test media, failed to affect glycolysis by mixed cultures of oral acidogenic organisms in these systems. The finding that manganese is a normal constituent of human whole saliva accords it the unique distinction of being the only bivalent ion as yet recovered from saliva to materially influence carbohydrate degradation by oral flora.

SUMMARY AND CONCLUSIONS

These studies show that the conversion of carbohydrates to acid residues by mixed oral acidogenic microorganisms in saliva and in bacteriologic media is inhibited by the presence of certain chelating compounds. The findings suggest that the inhibition results from the removal of Mn^{++} by the chelating compounds. This concept is supported by detailed knowledge of the mode of action of these chelators in other experimental systems.

Manganese was present in detectable quantities in all samples of stimulated human saliva tested for this element. The concentration ranged from 0.0011 to 0.017 μg per milliliter with a mean of 0.005 μg per milliliter.

These findings suggest that the manganese content of saliva is a factor in dental caries activity.

REFERENCES

1. Stephan, R. M.: In Vitro Studies of the Effects of Some Chemical Substances on the Growth of Oral Microorganisms and on Their Ability to Dissolve Tooth Salts, *J. D. Res.* **28**: 652, 1949.
2. Dreizen, S., Spies, H. A., Jr., and Spies, T. D.: The Copper and Cobalt Levels of Human Saliva and Dental Caries Activity, *J. D. Res.* **31**: 137, 1952.
3. Forbes, J. C., and Smith, J. D.: Studies on the Effect of Metallic Salts on Acid Production in Saliva. I, *J. D. Res.* **31**: 129, 1952
4. Bessman, S. P., and Doorenbos, N. J.: Chelation, *Ann. Int. Med.* **47**: 1036, 1957.
5. Albert, A., and Gledhill, W. S.: The Choice of a Chelating Agent for Inactivating Trace Metals. I. A Survey of Commercially Available Chelating Agents, *Biochem. J.* **41**: 529, 1947.
6. Fosdick, L. S., Hansen, H. S., and Epple, C.: Enamel Decalcification by Mouth Organisms in Dental Caries: Suggested Test for Caries Susceptibility, *J. A. D. A.* **24**: 1275, 1937.
7. Bentley, O. G., Snell, E. E., and Phillips, P. H.: A Microbiological Method for the Determination of Manganese, *J. Biol. Chem.* **170**: 343, 1947.
8. Gates, E. M., and Ellis, G. H.: Microcolorimetric Determination of Manganese in Biological Materials With 4,4′Tetramethyldiaminotriphenylmethane, *J. Biol. Chem.* **168**: 537, 1947.
9. Tittsler, R. P., Pederson, C. S., Snell, E. E., Hendlin, D., and Niven, C. F., Jr.: Symposium on the Lactic Acid Bacteria, *Bact. Rev.* **16**: 227, 1952.
10. MacLeod, R. A., and Snell, E. E.: Some Mineral Requirements of the Lactic Acid Bacteria, *J. Biol. Chem.* **170**: 351, 1947.
11. Carlson, W. W., Farina, L. V., and Whiteside-Carlson, V.: Manganese Antagonists in the Nutrition of *Lactobacillus acidophilus*, *J. D. Res.* **31**: 634, 1952.
12. Gale, E. F.: The Assimilation of Amino Acids by Bacteria. 8. Trace Metals in Glutamic Acid Assimilation and Their Inactivation by 8-Hydroxyquinoline, *J. Gen. Microbiol.* **3**: 369, 1949.
13. Albert, A., Rubbo, S. D., Goldacre, R. J., and Balfour, B. G.: The Influence of Chemical Constitution on Antibacterial Activity. Part III: A Study of 8-Hydroxyquinoline (Oxine) and Related Compounds, *Brit. J. Exper. Path.* **28**: 69, 1947.

OSTEOGENIC POTENTIAL OF CALCIUM HYDROXIDE AND OTHER MATERIALS IN SOFT TISSUE AND BONE WOUNDS

DAVID F. MITCHELL AND GOVIND B. SHANKWALKER

Indiana University School of Dentistry, Indianapolis, Ind.

IN THE course of a broad study of rat tissue reaction to various dental materials implanted subcutaneously,[1] it was noted that calcium hydroxide prompted the formation of calcified, bonelike tissue in the fibrous capsule around a pellet which had been implanted for 16 days. This study was devised to explore the osteogenic potential of this and other materials under similar circumstances, and then to test the osteogenic potential of this and other agents when implanted in surgical bone wounds of monkeys.

The search for a truly osteogenic substance has been in progress for many years.[2] Albee and Morrison[3] in 1920 reported that "Osmic acid, fibrin, blood, gelatine and lime salts, zinc chloride, thyroidin, glacial acetic acid, iodine tincture, adrenalin, hypophysis extract, bone marrow, copper sulphate, oil of turpentine, ammonia, lactic acid, silver nitrate solution, alcohol, carbolic acid, oak bark extract, vaccines and sera" had been used to stimulate bone growth without success. Many different calcium salts have been injected or implanted at fracture sites or in bone wounds[4] as well as in soft tissues[5] in the hope of stimulating osteogenesis, but with little consistent success. An extensive literature review[6] did not reveal any work of this nature using calcium hydroxide. This substance has been used for many years to cover pulp exposures, and a "dentin bridge" repair by the dental pulp beneath the material has been described. Barium sulfate also has been used for this purpose. It produced similar results in one limited study.[7] The other chemical compounds used in this study were chosen rather arbitrarily for comparison with calcium hydroxide.

A "despeciated" calf bone paste* has been suggested by some[8] as an aid to bone healing. "Anorganic bone"[9] also has been suggested as a grafting material. Plaster of Paris has been employed for similar purposes.[10, 11]

MATERIALS AND METHODS

A plastic pre-formed matrix was used to prepare small cylinders (2 by 2 mm.) of calcium hydroxide ($Ca[OH]_2$, pH 12.3) and water. After the paste was dried, small pellets of the material were implanted. Seventeen adult male white rats (Harlan, Indianapolis) were used. Two skin incisions were made on the backs of each of the anesthetized animals and the pellets were placed

This investigation was supported in part by Grant No. D600 from the National Institutes of Health, Public Health Service.

Received for publication June 12, 1958.

*Osteogen, E. R. Squibb & Sons, New Brunswick, N. J.

Fig. 1.—Tissues surrounding a pellet (*P*) of calcium hydroxide implanted for 16 days in the rat. Note the basophilic line (*B*) of heterotopic bone at the periphery.

Fig. 2.—Heterotopic bone (*B*) in the tissues around a pellet (*P*) of calcium hydroxide implanted in a rat for 20 days.

subcutaneously and the wounds closed with sutures. One animal was sacrificed at each of 17 intervals from 2 to 87 days and the tissue surrounding and including the implant was excised, fixed in formalin, embedded in paraffin, cut, and stained with hematoxylin and eosin. In some instances, additional sections were stained with von Kossa's silver nitrate stain and others with Gomori's trichrome stain.

Pellets made of calcium hydroxide and methyl cellulose paste* also were implanted in duplicate in 6 rats, and the tissue reaction was studied at 6 intervals ranging from 3 to 33 days. The following drugs (pH in parentheses) then were tested in duplicate in rats sacrificed after at least 3 varying periods, usually 4, 16, and 32 days: $Ba(OH)_2 \cdot 8H_2O$ (12.6); $Ca_3(PO_4)_2$ (10.9); MgO (10.3); $Mg(OH)_2$ (10.3); "anorganic bone" (8.5); calf bone paste (8.2); $Al_2O_3 \cdot H_2O$ (7.1); plaster of Paris (6.9); zinc oxide and eugenol (6.5); $BaSO_4$ (6.1); and $CaH_4(PO_4)_2 \cdot H_2O$ (3.5).

The tibias of 3 Cynomologous monkeys from South America were surgically exposed and 4 circular wounds, 6 mm. in diameter, were drilled through the cortex of the anterior portion of the shaft of each. One control wound in each tibia was not treated further. Each of the other wounds was filled with (a) calcium hydroxide and gelatin, (b) "osteogen," and (c) "anorganic" bone. After sacrifice or amputation, sections were made through the wounds at intervals of 44 days, 64 days, 77 days, and 7 months.

RESULTS

In the rats, a form of heterotopic ossification or calcification was found rather consistently in the soft tissues around the specimens of calcium hydroxide studied at 13 of the 17 intervals used. The earliest appearance was 10 days and most of the specimens taken from 10 to 35 days gave this reaction. In some cases, the calcified material closely resembled bone (Figs. 1 and 2). The material often fractured, presumably during sectioning, and it stained black by the selective von Kossa silver nitrate stain suggesting calcification. Around the early specimens there was considerable inflammatory reaction, which was replaced later by fibroplasia. Fat tissue often seemed to be associated with the "bony" deposit. The subdermal muscle, panniculus carnosus, often had been destroyed either by the operation incident to the implanting, or by the necrotizing action of the material. In several sections, it appeared that muscle bundles were partially calcified as a result of contact with the calcium hydroxide. Many sections made through the implants of calcium hydroxide and methylcellulose also demonstrated the induction of heterotopic bone.

Only 3 of the 11 other materials gave any evidence of calcification around them. It is difficult to ascribe any importance to the pH of the material tested. In only one instance barium sulfate induced a minimal amount of "bone" (20 days). Additional specimens of this material studied after 4, 10, 25, 32, and 39 days did not reveal any such reaction. Plaster of Paris induced "bone" in 8 of 15 specimens sectioned at 4 different intervals and will be

*Pulp-dent, Rower Dental Mfg. Co., Boston, Mass.

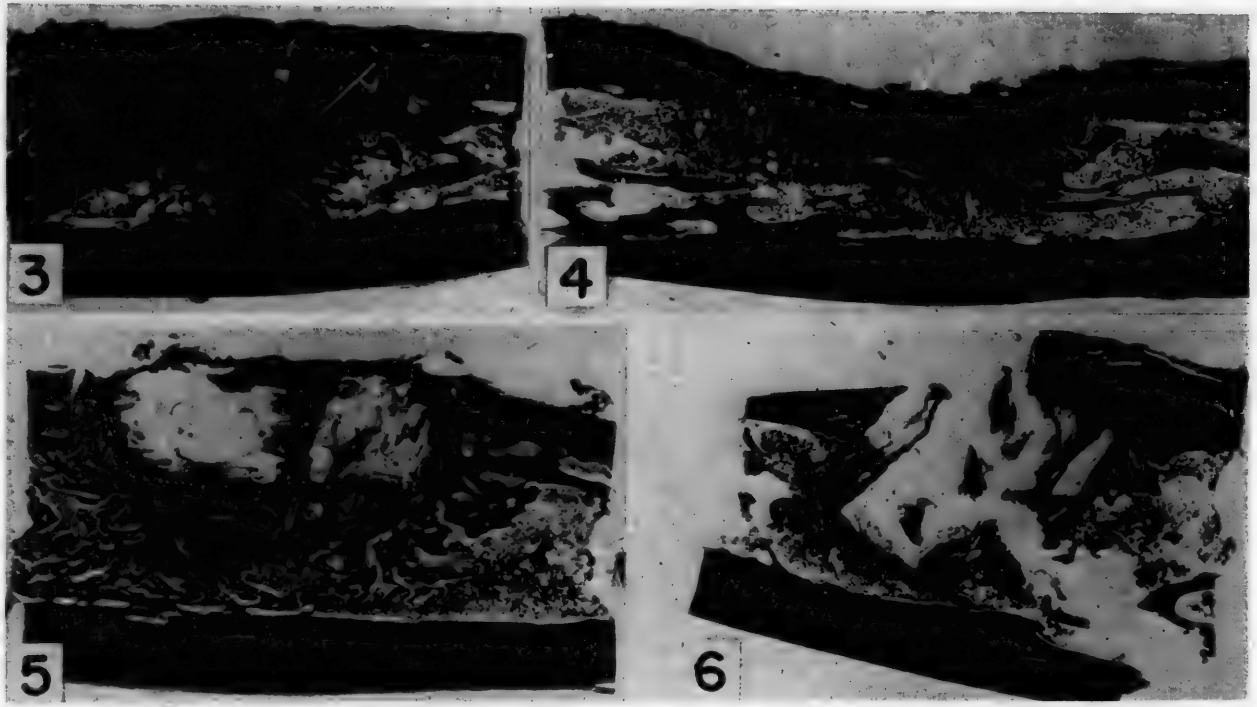


Fig. 3.—Longitudinal section through the center of a control, untreated wound of the monkey tibia after 44 days. Note the filling-in of the defect and underlying marrow space with new bone. The edges of the wound are marked with arrows.

Fig. 4.—This wound was treated with calf bone paste for 44 days. See the small pieces of calf bone surrounded by inflamed soft tissue. The new bone repair beneath the implant is apparent, but the diameter of the tibia is reduced in this region. The calcium hydroxide and gelatin-treated wounds were similar at 44 days.

Fig. 5.—This wound was filled with anorganic bone. The two square spaces were still filled with this fragile material after 44 days. The degree of organization of the spaces within the implant is not great. Surrounding new bone is comparable to that seen in the control section. (See Fig. 3.)

Fig. 6.—This tibia fractured at the site of implantation of a relatively large piece of anorganic bone (rectangular space) which was in place for 44 days. The amount of new bone repair around the implant was much less than may be seen in Figs. 3 and 5.

studied further. Magnesium hydroxide also induced "bone" in 2 of 6 specimens at 3 different intervals and further study is indicated. Specimens of anorganic bone and despeciated calf bone paste did not induce any apparent bone formation. There was a minimal inflammatory reaction to anorganic bone and it seemed to be quite inert even after being embedded for 65 days. The calf bone paste elicited considerably more inflammatory response around the 15-, 16-, and 27-day specimens. But those sectioned after 27 and 35 days were encapsulated with fibrous tissue and inflammation was negligible. Osteoclasts were frequently seen around the small pieces of calf bone at these later intervals, and the mass of paste seemed to be reduced in size. The tissue reaction to the paste which had been dried in air for 24 hours was quite comparable to that elicited by the fresh, refrigerated bone paste.

The tibial wounds of the monkeys furnished good comparative healing studies after the first interval of 44 days. The two control sections (Fig. 3) showed considerable filling-in of the defect with new bone at this stage. The calcium hydroxide-gelatin mixture, and calf bone paste caused an inflammatory and foreign body giant cell reaction, and delayed healing at this time (Fig. 4). The inflammatory reaction to anorganic bone implants was negligible and foreign body giant cells were less prominent; however, the material was not completely organized by connective tissue nor was there much new bone replacing this fragile material. One tibia was accidentally fractured through the anorganic bone-filled wound during the process of catching the monkey. The over-all effect of weakening of this stress-bearing bone due to the presence of the relatively inert anorganic bone is illustrated in Fig. 6.

In one tibia studied after 64 days, all wounds were well filled by bone regeneration, but microscopic pieces of the implanted materials were found in the periosteal tissues. It appears that these were extruded from the healing wounds. This leg also was accidentally fractured during observation, through the calcium hydroxide and gelatin-filled wound, so it was amputated and studied.

The other tibia of this monkey was studied after sacrifice of the animal at 77 days. The wounds were well healed in all cases. The only implanted material found consisted of remnants of anorganic bone incorporated into the surrounding new bone. A little fibrous tissue and a few giant cells had formed around this material.

After 7 months, the wounds were detectable in the tibias as illustrated in Fig. 7. Note the depression of the wounds filled with calf bone paste, and the elevated appearance of those filled with anorganic bone. These elevations were due to the presence of unresorbed implanted material. The control wounds and those treated with calcium hydroxide had healed to the same extent. Radiographs made at this time revealed all wounds apparently well filled in with bone, except for the anorganic bone-filled wounds which still showed the implanted material to be present. They also showed the same increased degree of radiopacity as was seen at the time of implantation. These findings were confirmed by the histologic sections. Remodeling of the shaft

in the areas of the control wounds and those treated with calcium hydroxide and gelatin, and calf bone paste was most nearly complete, whereas those treated with anorganic bone still contained much of this material and compensatory bone deposition in the original marrow space, comparable to that seen in Fig. 5, was still present.

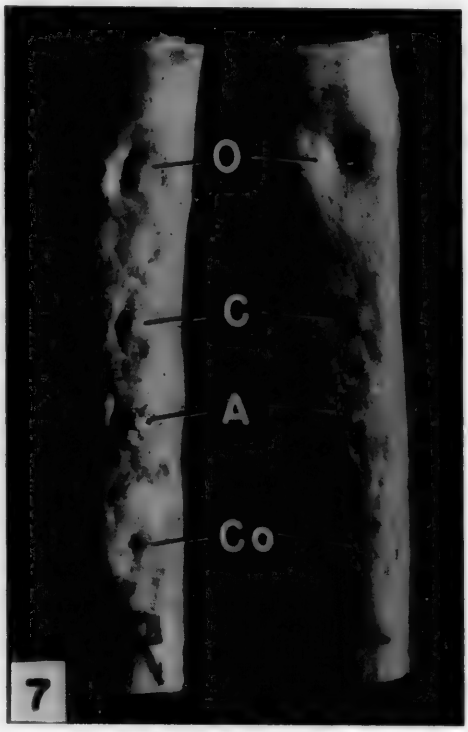


Fig. 7.—Gross appearance of the wounds 7 months after the operations. The circular depressions at the top (*O*) once were filled with calf bone paste. Below these are the barely distinguishable wounds treated with calcium hydroxide (*C*). Third from the top are anorganic bone-filled wounds (*A*) and the projections from the surface are particles of this material. The bottom wounds were untreated controls (*Co*).

CONCLUSIONS

Calcium hydroxide has a rather unique potential to induce the formation of heterotopic bone when implanted in the connective tissues of the rat. Of 11 other materials used in comparative studies, only plaster of Paris and magnesium hydroxide seem to warrant further study in this way.

Despeciated calf bone paste, and calcium hydroxide and gelatin mixtures appeared to delay the healing of small surgical wounds in monkey tibias. Anorganic bone is rather inert when implanted in the connective tissues of the rat, and in the surgical wounds of the tibia, and it alone remained unresorbed in the healed wounds at the 11-week and 7-month intervals. It is concluded that the untreated control bone wounds, as studied, healed better in all respects than did the treated bone wounds.

REFERENCES

1. Mitchell, D. F., and Amos, E. R.: Reaction of Connective Tissues of Rats to Implanted Dental Materials, I.A.D.R., 35th General Meeting, Atlantic City, March, 1957, Preprinted Abstracts, p. 59.
2. Ollier, L.: Traite experimental and clinique de la regeneration des os et de la production artificielle du tissue osseux, Paris, 1867, Victor Masson et Fils.
3. Albee, F. H., and Morrison, H. F.: Studies in Bone Growth. Triple Calcium Phosphate as a Stimulus to Osteogenesis, *Ann. Surg.* 71: 32, 1920.
4. Eden, R.: Untersuchungen über Vorgange bei der Verknöckerung, *Klin. Wchnschr.* 2: 1798, 1923.
5. Ray, R. D., and Ward, A. A.: A Preliminary Report on Studies of Basic Calcium Phosphate in Bone Replacement, Surgical Forum, Am. Coll. Surgeons, Philadelphia, 1952, W. B. Saunders Company, p. 429.
6. Shankwalker, G. B.: The Influence of Despeciated Calf Bone and Calcium Hydroxide on the Regeneration of Alveolar Bone in Dogs, Thesis, Indiana University, Dental Graduate School (Indianapolis, Ind.), November, 1957.
7. Via, W. F.: Barium Sulfate and Antibiotic Mixture in Pulpotomy, *J. D. Child.* 22: 195, 1955.
8. Tucker, E. J.: Studies on the Use of Cultured Calf Bone in Human Grafts, Clinical Orthopaedics, No. 7, Philadelphia, 1956, J. B. Lippincott Co., p. 171.
9. Losee, F. L., and Hurley, L. A.: Bone Treated With Ethylenediamine as a Successful Foundation Material in Cross Species Bone Graft, *Nature* 177: 1032, 1956.
10. Edberg, E.: Some Experiences With Filling Osseous Cavities With Plaster, *Acta chir. Scandinav.* 67: 313, 1930.
11. Peltier, L. F., Bickel, E. Y., Lilio, R., and Thein, M. S.: The Use of Plaster of Paris to Fill Defects in Bone, *Ann. Surg.* 146: 61, 1957.

CALCIFICATION AND ESTERASE LOCALIZATION IN DEVELOPING DENTIN

W. B. SAVCHUCK AND M. S. BURSTONE

National Institute of Dental Research, National Institutes of Health, Public Health Service, U. S. Department of Health, Education and Welfare, Bethesda, Md.

MICRORADIOGRAPHY is a valuable tool in indicating the sites of mineralization in developing teeth. The demonstration of esterase activity in the dentin of unerupted molars of newborn mice[1] and hamsters[2] prompted a study to determine whether a correlation exists between enzyme localization and the radiographic picture of calcification.

METHODS

Heads of 2- and 3-day-old hamsters were frozen and dried, then double embedded in celloidin and paraffin. Glass slides, ground at each end to produce roughened edges, were coated by dipping in a 2 per cent solution of a polyvinyl resin* in tetrahydrofuran. Both ends of the slides received a second dipping in the plastic to re-inforce the membrane. The undecalcified heads were sectioned at 8μ, and the sections were floated on the coated slides without albumin. The slides were then placed on a slide warming table heated to the melting point of the paraffin, after which they were dewaxed in xylene and transferred to alcohol. Esterase was demonstrated by the use of a simultaneous azo-dye coupling procedure employing naphthol AS-MX acetate (the acetate of the xylidide of 2-hydroxy,3-naphthoic acid).[2] Incubation time was one hour after which the slides were rinsed in distilled water. The plastic membrane was scored around each section and detached by flooding the section with water. After floating the section onto a specimen ring the membrane was pulled flat until dry. The soft x-ray apparatus was a Philips contact microradiograph. A Kodak 649-0 spectroscopic film was exposed for 2 minutes at 3 kilovolts and 3.2 milliamperes. Following the microradiography, the section was transferred to a glass slide by detaching the exposed area with an iris scalpel and mounting the section with a 20 per cent aqueous solution of hydroxyethyl cellulose.

RESULTS

Close agreement between histochemical and microradiographic findings was apparent where the stained section showed (1) a zone of calcified dentin devoid of stain, (2) a subjacent border of stained dentin in the process of

This paper was presented at the 36th General Meeting of the International Association for Dental Research, March 27-29, 1958, Detroit, Mich.

Received for publication June 16, 1958; revised by authors Sept. 9, 1958.

*Geon 400X110, B. F. Goodrich Chemical Co., Cleveland 15, Ohio.

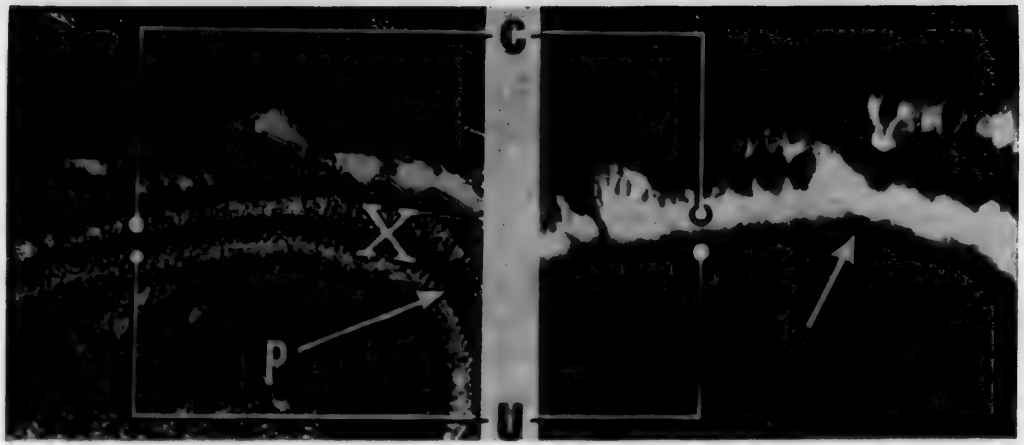


Fig. 1.—Lower first molar from a 3-day-old hamster showing esterase activity (*X*) in the stained section (left) corresponding with calcifying dentin (*arrow*) in the microradiogram (right). In the stained section, the arrow points from the pulp (*P*) to the predentin border which is radiolucent (black) in the radiogram. *C* and *U* designate the zones of calcified dentin and uncalcified predentin, respectively.

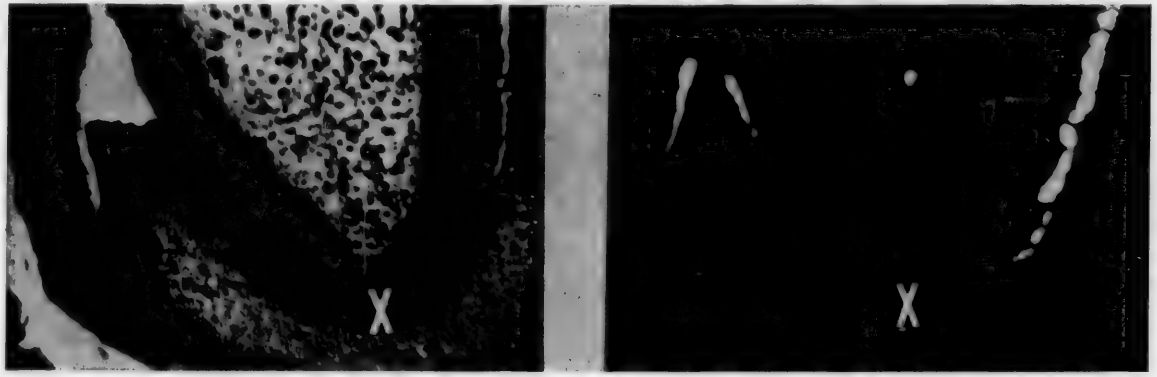


Fig. 2.—Lower first molar from a 2-day-old hamster. Region showing discontinuity of esterase activity (*X*) corresponds with region in microradiograph (right) showing complete absence of calcification.

calcifying, and (3) unstained, glassy predentin. Roentgenographically (Fig. 1) the calcified dentin appeared white and the predentin appeared radiolucent while the esterase-active, calcifying dentin was discernible as a gray border interposed between the opaque (white) dentin and the radiolucent (black) predentin.

In regions where the dentin of the molar tooth germ had not begun to calcify (Fig. 2), the esterase stain was absent, coinciding roentgenographically with a discontinuity of the calcifying dentin.

Attempts to correlate microradiographic findings with sites of alkaline phosphatase activity were unsuccessful.

SUMMARY

Hamster teeth were studied by a coupling of technics, the same section being microradiographed after the histochemical procedure. Microradiographic findings corroborated previous reports of the occurrence of esterase activity in areas where dentin was undergoing calcification.

REFERENCES

1. Burstone, M. S.: Esterase Activity of Developing Bones and Teeth, A. M. A. Arch. Path. 63: 164, 1957.
2. Burstone, M. S., and Keyes, P. H.: Studies on Calcification. I. The Effect of Inhibition of Enzyme Activity on Developing Bone and Dentin, Am. J. Path. 33: 1229, 1957.

Erratum

In the article entitled "Calcification. XVI. Composition of Bones and Teeth in Relation to Blood and Diet in the Cotton Rat," by Albert E. Sobel and Albert Hanok, pages 631-637 of the August, 1958, issue of the JOURNAL, the percentage Ca of Basal I given in the first line of Table II should be 0.0286.

RESEARCH ANNOTATION*

ORAL LACTOBACILLUS ACIDOPHILUS GROWTH INHIBITING ACTIVITY OF HUMAN GLOBULIN

THOMAS L. GRISAMORE AND PATRICK D. TOTO

Loyola University School of Dentistry, Chicago 12, Ill.

FOLLOWING a report (Green, G. E., *Bact. Proc.*, Society of Am. Bacteriologists, Chicago, 1958, p. 87) of globulin-like activity in the saliva of caries-immune individuals, this study of the effects of various globulin fractions on the growth of *Lactobacillus acidophilus* was carried out.

A strain of *L. acidophilus*, from a clinically positive case of caries, was isolated on Rogosa medium, subcultured on the same medium, and incubated for 72 hours. A suspension of the growth was made in distilled water, and a 1:10 dilution of this made in physiologic saline. One milliliter and 0.1 ml. of the saline-organism dilution were placed into various human or bovine globulin-modified (Rogosa) media. The quantity of globulin used was equivalent to that found in normal human and bovine blood plasma.

The modifications were as follows:

Globulin Fraction†		*Rogosa Medium*
H_{II} —0.33 Gm.	to	100 ml.
H_{III}—1.0 Gm.	to	100 ml.
H_{IV_4}—0.5 Gm.	to	100 ml.
B_{II} —0.33 Gm.	to	100 ml.
B_{III}—1.0 Gm.	to	100 ml.
B_{IV_4}—0.5 Gm.	to	100 ml.

The following protocol illustrates the design of the experiments and the results obtained:

GLOBULIN FRACTION	SALINE DILUTION OF L. ACIDOPHILUS SUSPENSION	GROWTH	GLOBULIN FRACTION	SALINE DILUTION OF L. ACIDOPHILUS SUSPENSION	GROWTH
H_{II}	1:10	–	B_{II}	1:10	+
H_{II}	1:100	–	B_{II}	1:100	+
H_{III}	1:10	+	B_{III}	1:10	+
H_{III}	1:100	+	B_{III}	1:100	+
H_{IV_4}	1:10	+	B_{IV_4}	1:10	+
H_{IV_4}	1:100	+	B_{IV_4}	1:100	+
Control	1:10	+	H_{II}	Control organism	+
Control	1:100	+	H_{II}	Control organism	+

Discussion.—The H_{II} globulin fraction is predominantly gamma globulin, the antibody containing fraction of serum, and although H_{II} and B_{II} are found in the same concentration in the respective plasmas, only H_{II} inhibited the growth of *L. acidophilus*. Moreover, a test utilizing H_{II} globulin fraction and a control organism showed no inhibition. The findings suggest that there are present, in human globulin fraction II, antibodies which inhibit the growth of *L. acidophilus*.

Received for publication Oct. 3, 1958.

*Short notes with or without illustrations submitted to this section are reviewed and published promptly on one page of the JOURNAL in accordance with the previously announced editorial policy (*J. D. Res.* 37: 373, 1958).

†H_{II}, H_{III}, and H_{IV_4} are human globulin fractions. B_{II}, B_{III}, and B_{IV_4} are bovine globulin fractions. Furnished by Dr. H. J. Antonides of the Armour Pharmaceutical Company, Kankakee, Ill.

SUPPORTING ASSOCIATES*

OF

THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

AND

THE JOURNAL OF DENTAL RESEARCH

The Acralite Company, Inc., New York, N. Y.

Julius Aderer, Inc., New York, N. Y.

The Amalgamated Dental Company, Ltd., London, Eng.

Baker and Company, Newark, N. J.

Bristol-Myers Company, New York, N. Y.

L. D. Caulk Company, Milford, Del.

Colgate-Palmolive Company, Jersey City, N. J.

Crescent Manufacturing Company, Chicago, Ill.

Dental Materials' Group; I.A.D.R., Indianapolis, Ind.

Dentists' Supply Company of New York, York, Pa.

Hanau Engineering Company, Inc., Buffalo, N. Y.

J. F. Jelenko & Company, Inc., New York, N. Y.

H. D. Justi & Sons, Inc., Philadelphia, Pa.

Kerr Manufacturing Company, Detroit, Mich.

Precious Metals Research Works, Inc., New York, N. Y.

Procter & Gamble Company, Cincinnati, Ohio

Squibb Institute for Medical Research, New Brunswick, N. J.

Ticonium Division, Consolidated Metal Products Corp., Albany, N. Y.

The S. S. White Dental Manufacturing Company, Philadelphia, Pa.

*Supporting Associates have made contributions to the Association for support of the JOURNAL. These contributions are in multiples of one hundred dollars.

INDEX

Volume 37, 1958

ABRASION, tooth, measurement of, by dentifrice, utilizing radioactive teeth, 1060

Abstracts, read-by-title,* 82, 964

Acid drinks, effect on molar teeth of rats, 752 (Abs.)

formation, effect of peptides on, in sugar-saliva mixtures, 73 (Abs.)

rate of, effect of saliva on, in plaques, 73 (Abs.)

Acrylic and porcelain pontics, tissue changes under, 66 (Abs.)

Adhesion of foodstuffs to teeth, 72 (Abs.)

AFONSKY, D., 59 (Abs.)

ALBRIGHT, J. T., 18 (Abs.)

Alkaline phosphomonoesterase, distribution in the developing human tooth, 758 (Abs.)

ALLAN, J. H., 750 (Abs.)

Alligator mississippiensis Daud. II. dentin development, 532

III. enamel development, 540

ALLISON, J. R., 66 (Abs.)

Alloxan injections, influence on development of caries in rat, 755 (Abs.)

Alloys, dental, tarnish of, 837

ALLRED, H., 751 (Abs.)

Alpha-tocopherol and protein, curative effect on incisor of vitamin E-depleted rats, 732

Amalgam restorations, analysis of stresses, 516

silver, study of fatigue characteristics, 136

Amino-acid decarboxylases in sediment from incubated saliva, 30 (Abs.)

Ammonia and volatile amines, production of, in saliva, 30 (Abs.)

Amplifier-graphic recorder for measuring enamel permeability, 23 (Abs.)

Amylase, effect of ingested carbohydrates on concentrations, 755 (Abs.)

Anachoresis, study of, utilizing *Serratia marcescens*, in mice, 42 (Abs.)

ANDERSON, D. J., 312, 669

ANDERSON, D. L., 42 (Abs.)

ANDERSON, T. R., 96 (Abs.)

Anesthesia, local, determination of efficiency, 13 (Abs.)

Anesthetic needles, contamination of, 144

Antarctica, dental observations while wintering in, 619

Antibiotics, effect of hydrocortisone and poly—, on rat molar pulp, 886

in vitro effects on oral spirochetes, 11 (Abs.)

therapy, effect on salivary hyaluronidase. VI, 453

Anti-cariogenic agents, solubility of intact enamel surfaces treated with, 412

Apatite, nature of water on surface, 25 (Abs.)

APPLEBY, R. C., 44 (Abs.)

APPLEMAN, M. D., 427, 527

ARMSTRONG, W. D., 69 (3 Abs.)

ARMSTRONG, W. G., 1001, 1016

ARNIM, S. S., 45 (Abs.)

Articulators, protrusive movement in, 125

Ascorbic acid, role in fluoride retention, 72 (Abs.)

Automatic shot loading machine for transverse tests on denture base resins, 87 (Abs.)

Autoradiographs of solder joints with fluorine-18, 96 (Abs.)

AVERY, J., 7 (Abs.), 14 (Abs.), 46 (Abs.), 79 (Abs.), 81 (Abs.), 880

AYERS, H. D., 96 (Abs.), 368

BACHEM, E., 54 (Abs.), 55 (Abs.), 485, 824

Bacteremia associated with dental procedures in severely handicapped children, 12 (Abs.)

Bacteria, branching, occurrence in root canal cultures, 754 (Abs.)

effect of secretions of human submaxillary and parotid glands on, 756 (Abs.)

presence in healthy gingival sulcus, 288

Bacterial chondrosulfatase, spreading factor activity of, 48 (Abs.)

*All published articles and abstracts actually read at meetings are indexed here under subject and author entries. Abstracts read by title have been listed in the following ways: (1) J. D. RES. 37: 986, 1958, complete abstracts and author index; (2) 37: 82, 1958, title only. Read-by-Title abstracts are not included in this index.

Bacterial—Cont'd
enzymes and periodontal disease, 100
hydrolysis of chondroitin sulfate, 623
localization in pulps of rat molars following bacteremias, 67 (Abs.)
Bacteriologic census of human saliva, 8 (Abs.), 697
status of periapical tissue in infected pulpless teeth, 67 (Abs.)
Baer, P. N., 61 (Abs.)
Bahn, A. N., 48 (Abs.)
Barley and fish diet, effect on caries in rat, 419
Barnard, P., 33 (Abs.), 34 (Abs.)
Barnhart, G. W., 948 (Annot.)
Barr, J. H., 14 (Abs.)
Bartels, H. A., 50 (Abs.), 654
Bates, J. F., 755 (Abs.)
Bavetta, L. A., 6 (Abs.), 16 (Abs.)
Baxter, G. I., 51 (Abs.)
Bayne, D. I., 73 (Abs.)
Beck, D. J., 73 (Abs.)
Beck, J. O., 41 (Abs.)
Becks, H., 195
Belanger, L. F., 264
Bell, W. H., 45 (Abs.)
Bender, I. B., 678
Bennett, D. T., 144
Bentley, K. D., 33 (Abs.)
Berk, H., 66 (Abs.)
Berman, D. S., 229
Bernick, S., 6 (Abs.), 16 (Abs.), 61 (Abs.)
Bernier, J. L., 21 (Abs.), 94 (Abs.)
Berzinskas, V. J., 31 (Abs.)
Beta-aminopropionitrile, effect on wound healing, 38 (Abs.)
Bevelander, G., 6 (Abs.)
Bhaskar, S. N., 21 (Abs.), 467
Bhatia, H. L., 66 (Abs.)
Bhussry, B. R., 832, 1045, 1054
Bibby, B. G., 73 (Abs.), 570
Bickford, R. G., 13 (Abs.)
Bien, S. M., 96 (Abs.), 368
Binkley, F., 623
Bishop, J. G., 80 (Abs.)
Bixler, D., 54 (Abs.), 56 (Abs.), 407, 410, 649, 848
Blackwell, R. Q., 23 (Abs.), 30 (2 Abs.), 73 (Abs.)
Blackwood, H. J. J., 753 (Abs.)
Blechman, H., 50 (Abs.), 77 (Abs.), 654
Blood constituents, correlation with periodontal condition, 63 (Abs.)
flow in mandibular artery of anesthetized dogs, 80 (Abs.)
to the teeth, alteration of. II. effect on caries and salivary gland structure and function in the rat, 1069
Blood—Cont'd
plasma, protein fractions in, and in dental pulp fluid, 66 (Abs.)
and serum, analysis of fluoride, 69 (Abs.)
Bock, R. M., 1123
Boeke, P. R., 65 (Abs.)
Bogoroch, R., 458
Bolden, T. E., 54 (Abs.)
Bone, anorganic, implants of, histologic comparison with bovine implants in human being, 45 (Abs.)
response of oral tissues to heterologous grafts of, 44 (Abs.), 45 (Abs.)
carbonate content, 26 (Abs.)
effect of diet on, 6 (Abs.)
enamel and dentin solubility in acetate, lactate and aspartate buffers, 57 (Abs.)
Bones and teeth, composition of, in relation to blood and diet in the cotton rat, 631
Borden, J. V., 94 (Abs.)
Bose, A. K., 23 (Abs.)
Bouchal, A. W., 74 (Abs.)
Boult, E. H., 760 (Abs.)
Bowen, R. L., 90 (Abs.)
Bowen, W. H., 9 (Abs.)
Boyd, W. S., 288
Boyne, P. J., 44 (Abs.), 45 (Abs.)
Bradford, E. W., 754 (Abs.)
Braley, E. M., 65 (Abs.)
Branchial cysts, histogenesis of, 21 (Abs.)
Brauer, G. M., 86 (Abs.), 87 (Abs.), 89 (Abs.), 547
Brewer, H. E., 23 (Abs.), 1069
British division IADR, proceedings, 748
Brodie, A. G., 467
Broge, R. W., 638, 1060
Brown, H. F., 26 (Abs.)
Brown, L. R., 59 (Abs.)
Brudevold, F., 25 (2 Abs.), 26 (Abs.), 60 (Abs.), 254, 318, 991
Bulat, T. J., 43 (Abs.)
Buonocore, M., 57 (2 Abs.)
Burgess, R. C., 42 (Abs.)
Burke, G. W., 67 (Abs.)
Burlington Orthodontic Centre, reliability of measurements from cephalograms at, 853
Burnett, G. W., 581, 590, 605
Burns, C. L., 89 (Abs.)
Burrill, D. Y., 51 (Abs.), 63 (Abs.)
Burrows, L. R., 40 (Abs.)
Burs, design factors related to cutting effectiveness, 91 (Abs.)
Burstone, C. J., 80 (Abs.)
Burstone, M. S., 5 (2 Abs.), 1164
Butcher, E. O., 35 (Abs.), 62 (Abs.), 384

BUTTNER, G., 326, 412
BUTTNER, W., 58 (Abs.), 419, 860

CALCIFICATION, enzyme patterns associated with, 5 (Abs.)
and esterase localization in developing dentin, 5 (Abs.), 1164
of tooth germs in vitro, experimental studies, 738
tooth, sex difference, 561
Calcified tissues, structure, internal, 7 (Abs.), 880
moisture content, 581
Calcium hydroxide and other materials, osteogenic effect in wounds, 1157
effect on unexposed pulp, 758 (Abs.)
phosphate salts, effect on caries, 860
and phosphorus metabolism in *ia* rat, 432
reduction, effect on skeletal development in *Echinarachnius parma* embryos, 6 (Abs.)
Calculus, composition of, some observation on, 749 (Abs.)
formation, characteristics of a bovine submaxillary gland extract used for, 77 (Abs.)
from a substrate containing a bovine submaxillary gland extract, 78 (Abs.)
occurrence of whitlockite in, 749 (Abs.)
CALDWELL, R. C., 72 (Abs.), 301
Carbonydrate components of dental tissue, 42 (Abs.)
effect on salivary gland function, 53 (Abs.)
injected, and pyridoxine, effect on caries and dentinal circulation, 874
injections, intraperitoneal, and caries in suckling rats, 719
Carbonate, content of bones and teeth, 26 (Abs.)
Carbon dioxide, inorganic in enamel, 991
Carcinogenesis, effect of vitamin A deficiency on, 19 (Abs.)
in duct-ligated submaxillary salivary glands, 19 (Abs.)
oral, effect of topical application of carcinogens on palatal mucosa of hamsters, 19 (Abs.)
experimental, further studies on, 18 (Abs.)
histochemical observation of basement membrane changes in, 17 (Abs.)
Caries activity and pH, titratable alkalinity and rate of flow of parotid saliva, 906
influence on respiration of salivary microbial flora, 43 (Abs.)
beginning use of vital stains as indicators, 60 (Abs.)
changes induced in cariogenicity of diets by interchanging salt mixtures, 33 (Abs.)
Caries—Cont'd
in children, effect of sodium N-lauroyl sarcosinate on, 75 (Abs.)
and dentinal circulation, effect of pyridoxine and injected carbohydrate, 874
destruction, 754 (Abs.)
early lesions in enamel, 751 (Abs.)
effect of fluoride and nonfluoride containing tin salts in children, 422
levels in salivariadenectomized rats, 170
modified stannous fluoride-calcium pyrophosphate dentifrice, 448
topical potassium fluorostannite, 417
stannous fluoride in adults, 415
effects of feeding calcium phosphate salts, 860
of enamel, 59 (Abs.)
experience in 200 children, dietary habits and, 76 (Abs.)
and hyaluronidase activity of saliva, 114
and the oral environment, 75 (Abs.)
and salivary sodium, potassium and chloride levels, 401
hyaluronidase activity and caries susceptibility tests, 306
experimental, effect of platinic chloride, 34 (Abs.)
human foods, 865
influence of extraction of opposing molars, 53 (Abs.)
production of, in Egyptian rodents, 31 (Abs.)
fissure, some histological findings in, 751 (Abs.)
hamster, influence of nongenetic factors in transmission of, 32 (Abs.)
inhibitory agents, the clinical dental trial, 335
in vitro, studies by microhardness tests, 301
lactobacillus counts, 50 (Abs.)
preliminary interrelational study of in 1,800 adult patients, 77 (Abs.)
quantitative assessment of radiodensity and birefringence in incipient lesions, 60 (Abs.)
rat, effect of $CaHPO_4$ dietary supplement, 34 (Abs.)
effect of diet of barley and fish, 419
of metal salts of EDTA, 32 (Abs.)
of pilocarpine, 410 (Abs.)
of selenium salts, 35 (Abs.)
of trace element sea salt mixture, 33 (Abs.)
high sucrose diet during calcification of teeth, 53 (Abs.)
influence of alloxan injections on development of, 755 (Abs.)
initiation and promotion of, 31 (Abs.)
intraperitoneal carbohydrate injections, 719 (Abs.)

Caries, rat—Cont'd
molar, method for diagnosis and scoring, 1088
pattern induced by high carbohydrate, low fat diets, 1077
occlusal, effect of sugar in various forms, 570
relation of feeding regimens, 52 (Abs.)
salivary flow and food consumption, 407
gland extirpation and duct ligation, 54 (Abs.)
systemic etiological factors of, 32 (Abs.)
susceptibility tests, salivary hyaluronidase activity and dental caries experience, 306
and thyroid activity, in mouse, 451
Cariogenic diets, studies on heat, treatment of, 52 (Abs.)
effect of addition of sugar cane syrup to diet of rats, 758 (Abs.)
Cariogenicity of Emory and Harvard diets, studies on, 33 (Abs.)
Carious and sound dentin, action of collagenase, 1001
lesions, progression of, by surfaces, 75 (Abs.)
CARLSON, C., 69 (Abs.)
CARTER, W. J., 13 (Abs.), 63 (Abs.), 79 (Abs.), 902
Cartilage, limulus, comparative studies of calcified and skeletal tissues, 4 (Abs.)
CASTALDI, C. R., 12 (Abs.)
Casting shrinkage of cobalt-chromium alloys, 94 (Abs.)
CATCHPOLE, H. R., 39 (Abs.)
CATTONI, M., 767
CAUL, H. J., 87 (Abs.)
CAVALLARO, J., 50 (Abs.), 654
Cavity formation, role of psychologic predisposition in, 330
preparation, cavity roughness, effects of on adaptation of gold castings, 95 (Abs.)
clinical evaluation of modern instrumentation for removal of tooth structure, 93 (Abs.)
full crown preparations, early pulp changes in teeth of a dog, following, 220
high speed rotary operative dental procedures. I. tooth volume and pulpal pathology, 67 (Abs.)
human pulpal reactions following, 68 (Abs.)
symposium on modern methods of removal of tooth structure, 94 (Abs.)
varnish, penetration of, by phosphoric acid, 43 (Abs.)
CECIL, H. C., 38 (Abs.)
Cementum, continuous apposition of, 44 (Abs.), 1035
determination of thickness of, 44 (Abs.)
optical and electron microscopy of, observations, 760 (Abs.)
Cephalograms, at Burlington Orthodontic Research Centre, reliability of measurements, 853
natural head position, prerequisite for satisfactory interpretation of, 15 (Abs.)
serial, study of mandibular movement in children, 15 (Abs.)
CHALUP, M. J., 214
CHARBENEAU, G. T., 95 (Abs.)
CHAUDHRY, A. P., 22 (Abs.)
CHAUNCEY, H. A., 28 (Abs.), 29 (2 Abs.), 906
Chelating agents and *o*-ethoxybenzoic acid, reaction with metal oxides, 547
Chicago Section, IADR Scientific Programs, 762
CHILTON, N. W., 335, 444
CHIPMAN, A. D., 93 (Abs.)
Chloride levels in parotid secretion, 902
Chondroitin sulfate, bacterial hydrolysis, 623
Chromatography, vapor phase, analysis of methyl-methacrylate co-polymers, 86
CLARK, H. B., JR., 45 (Abs.)
CLARK, P. G., 848
CLEMENTS, W. G., 31 (Abs.)
Cleft palate, intelligence quotient and congenital, 79 (Abs.)
possible etiological factor in, 79 (Abs.)
Clinical dental trial. I. new caries-inhibiting agent, 335
II. relaxing drug for operative dentistry, 444
Cobalt-chromium alloys, casting shrinkage of, 94 (Abs.)
Cobalt-induced polycythemia, 1141
COHEN, A. H., 107, 114, 306, 453
COHEN, G., 67 (Abs.)
COHEN, M. M., 76 (Abs.)
COLBY, R. A., 7 (Abs.)
Collagen, metabolic significance of, in tooth structure, 276
Collagenase, action on sound and carious dentin, 1001
resistant forms, modification of organic matrix of dentin to, 1016
Color of silicate cements, standardization of, 89 (Abs.)
vision and shade matching, defective, in dentistry, 760 (Abs.)
COMAR, C. L., 264
Composition of teeth, human, 590
Computing mean age of eruption and associated statistics, procedures for, 938

Condylar cartilage, vascularization of, 753 (Abs.)
Connective tissue, colloids of, 39 (Abs.)
function in health and disease, 40 (Abs.)
Contamination of anesthetic needles, 144
Contour meter, 88 (Abs.)
COOKE, C., 753 (Abs.)
COOLEY, W. E., 24 (Abs.)
COPELAND, P. L., 91 (Abs.)
CORNELL, J. A., 86 (Abs.), 87 (Abs.)
Corrosion, investigation of certain dental materials, 88 (Abs.)
Cortisone, infection and, 11 (Abs.)
injected mice, histochemical study of salivary glands, 119
COSTICH, E. R., 7 (Abs.), 36 (2 Abs.), 46 (Abs.), 880
Cotton rat, composition of bones and teeth in relation to blood and diet, 631
COX, G. J., 31 (Abs.)
CRABB, H. S. M., 750 (Abs.)
CRAIG, R. G., 91 (Abs.), 661, 710
Craniometric and dental investigation of Romano-British skulls and jaws, 753 (Abs.)
CUMMINS, N., 69 (Abs.)
CURBY, W. A., 17 (Abs.), 76 (Abs.)
CURWEN, M. P., 669

DAHLBERG, A. A., 1123
DAHLIN, D. C., 21 (Abs.)
DARLING, A. I., 749 (Abs.), 751 (Abs.)
DASLER, W., 492
DAVIES, G. N., 570
DAVIES, T. G. H., 758 (Abs.)
DAVIS, G. H., 73 (Abs.)
DAVIS, G. H. G., 10 (Abs.)
DEGLER, R. L., 29 (Abs.)
DEGOOYER, J. J., 43 (Abs.)
DEMENT, J. A., 24 (Abs.)
Dental evaluation of 200 public school children in Salem, Mass., 76 (Abs.)
materials, investigation of corrosion of, 88 (Abs.)
papilla in tissue culture, 767
Dentin, destruction by caries, 754 (Abs.)
developing, calcification and esterase localization, 5 (Abs.), 1164
development of, in *Alligator mississippiensis* Daud, 532
differential staining of undecalcified sections, 751 (Abs.)
effect of CO_2 toxicity on formation of, 7 (Abs.)
elastic and mechanical properties, 710
and enamel, and bone solubility in acetate, lactate and aspartate buffers, 57 (Abs.)
effect of certain acid anions on solubility of, 57 (Abs.)
microhardness of, 91 (Abs.)
fluoride content, 25 (Abs.)
microbial decomposition of enamel and, in vitro, 48 (Abs.)
Dentin—Cont'd
organic matrix, modification of, to collagenase-resistant forms, 1016
peritubular matrix of, 7 (Abs.)
sensitivity, 669
solubility, effect of certain restorative materials, 811
sound and carious, action of collagenase, 1001
Dentistry, analgesic agents in, 13 (Abs.)
Denture adaptation, evaluation of, 88 (Abs.)
base materials, physical properties of, 87 (Abs.)
reliners, physical properties of, 87 (Abs.)
resins, transverse tests of, automatic shot loading machine for, 87 (Abs.)
reliners, composition of, used by public, 87 (Abs.)
DERKSEN, A. A. D., 125
DEUSCHLE, F. M., 8 (Abs.)
Development of tooth buds cultured in vitro, 1100
DEWAR, M. R., 100
DICKSON, G., 89 (Abs.)
Diet, $CaHPO_4$ supplement and experimental caries in rat, 34 (Abs.)
cariogenic, studies on heat treatment of, 52 (Abs.)
cariogenicity of, changes induced in, by interchanging salt mixtures, 33 (Abs.)
synthetic amino acid, effect on bones and teeth, 6 (Abs.)
Dietary habits and dental caries experience in 200 children, 76 (Abs.)
utilization of fluorine in presence of calcium salts, 70 (Abs.)
Dimensional change in wax patterns during setting of gypsum investments, 351
DISRAELY, M., 50 (Abs.)
DIXON, A. D., 753 (Abs.)
Domesticated animals, oral and pharyngeal pathology of, 22 (Abs.)
DONER, A., 63 (Abs.)
DORMAN, H. L., 80 (Abs.)
DRAUS, F. J., 77 (Abs.), 78 (Abs.)
DREIZEN, J. G., 1149
DREIZEN, S., 1149
DRESSER, W. J., 45 (Abs.)
DUTE, H. L., 17 (Abs.)

EARLE, L., 623
EARNSHAW, R. G., 91 (Abs.), 94 (Abs.)
Echinarachnius parma embryos, effect of Ca reduction on skeletal development, 6 (Abs.)
Editorial Views and News, 373, 569, 766, 989
Editor's Viewpoint, 1, 183
Effect of inorganic ions on utilization of fluoride in rats, 70 (Abs.)
EID, C. N., 1141

Elastic impression materials, determination of shrinkage, 92 (Abs.)
Electromechanical dental model surveyor, 90 (Abs.)
Electron microscopic observations, enamel effects of stannous fluoride and sodium fluoride, 638
studies of early stages of amelogenesis, 22 (Abs.)
study of anorganic enamel, 23 (Abs.)
microscopy, study of particulates in periodontal exudate, 51 (Abs.)
and optical microscopy of hman cementum, observations, 760 (Abs.)
study of submicroscopic structure of human enamel, 243
Electrophoresis of saliva, 26 (Abs.)
Ellison, S. A., 28 (Abs.)
El Tannir, M. D., 88 (Abs.), 837
Elzay, R. P., 53 (Abs.)
Emergence of permanent teeth in Pima Indian children, 1123
Emory and Harvard diets, cariogenicity of, 33 (Abs.)
Enamel, anorganic, electron microscopic study, 23 (Abs.)
application of tin and fluoride, 24 (Abs.)
carbonate, effect of fluoride incorporation and acid treatment, 26 (Abs.)
caries, 59 (Abs.)
changes in microhardness following treatment with lactate buffers, 56 (Abs.)
chemical and morphologic factors in susceptibility to acid attack, 59 (Abs.)
and physical studies. I. specific gravity and nitrogen content of different surfaces, 832
II. specific gravity, nitrogen content, and hardness of discolored enamel, 1045
III. specific gravity, nitrogen content and histologic characteristics of opaque white enamel, 1054
density, 23 (Abs.)
dentin and bone solubility in acetate, lactate and aspartate buffers, 57 (Abs.)
and dentin, effect of certain acid anions on solubility, 57 (Abs.)
microhardness, 91 (Abs.), 661
development, electron microscopic studies of early stages of amelogenesis, 22 (Abs.)
in *Alligator mississippiensis* Daud. III., 540
distribution of fluoride in relation to fluoride in drinking water, 318
early lesion of caries, 751 (Abs.)
effects of salivary fluoride on solubility, 58 (Abs.)
of stannous fluoride and sodium fluoride, electron microscopic observation, 638
Enamel—Cont'd
fluoride content, 25 (Abs.)
inorganic carbon dioxide, 991
lamellae, distribution of, in longitudinal plane, 750 (Abs.)
microbial decomposition of, and dentin in vitro, 48 (Abs.)
morphology, effect of, in susceptibility to bacterial attack, 59 (Abs.)
permeability, measurement with amplifiergraphic recording method, 23 (Abs.)
powder, preparation in water, 748 (Abs.)
powdered, effect of temperature on solubility reduction by sodium N-palmitoyl sarcosinate, 74 (Abs.)
identification of reaction products of stannous fluoride and sodium fluoride with, 58 (Abs.)
removal of fluoride from solution of sodium and stannous fluoride by, 748 (Abs.)
solubility rate in acid, 57 (Abs.)
studies on mineralization pattern of, 750 (2 Abs.)
study of submicroscopic structure by electron microscopy, 243
of surface reaction to various polishing agents, 91 (Abs.)
surface and subsurface, natural fluoride content and solubility rate, 254
surfaces, intact, solubility and potential anticariogenic agents, 412
Engel, M. B., 39 (Abs.), 40 (Abs.)
Englander, H. R., 13 (Abs.), 28 (Abs.), 29 (Abs.), 50 (Abs.), 73 (Abs.), 902, 906
Enright, E. T., 14 (Abs.)
Enzymes, bacterial, and periodontal disease, 100
effect on fixed human gingiva, 63 (Abs.)
hydrolytic, sources, and periodontal disease, 63 (Abs.)
patterns associated with calcification, 5 (Abs.)
Epithelium, role of, in experimental extraction wounds, 187
Ershoff, B. H., 6 (Abs.), 16 (Abs.)
Eruption, first permanent molars, progress of, 82 (Abs.)
mean age and associated statistics, procedures for computing, 938
Eruptive mechanism and movement in rat first molar, 467
Esterase and calcification localization in developing dentin, 5 (Abs.), 1164
Everett, F. G., 162
Exfoliative cytology, a diagnostic aid for detection of oral neoplasms, 20 (Abs.)
Experimental animals, *see* Individual animal
production of carcinoma of tongue in mice, 950 (Annot.)
Extraction wounds, experimental, role of epithelium, 187

Fairhurst, C. W., 88 (2 Abs.), 96 (Abs.)
Fanning, E. A., 4 (Abs.)
Farber, S. M., 195
Farrell, J., 760 (Abs.)
Fath, E. H., 70 (Abs.)
Fertig, J. W., 335, 444
Fessenden, E., 78 (Abs.)
Fiegel, L. J., 91 (Abs.)
Filling materials, penetration of isotopes, 17 (Abs.)
Film thickness, limiting factor in chemical inhibition of glycolysis, 798
influence of, on resistance to glycolysis inhibition, 74 (Abs.)
Fine, A., 4 (Abs.), 62 (Abs.), 292
Fischer, R. B., 23 (Abs.)
Fisher, A. K., 65 (2 Abs.), 214
Fisher, B., 64 (Abs.), 1115
Fitzgerald, R. J., 11 (Abs.), 52 (Abs.)
Flanagan, V. D., 37 (Abs.), 39 (Abs.), 47 (Abs.), 49 (Abs.), 80 (Abs.)
Fleming, H. S., 37 (Abs.)
Fluoride, analysis of, in plasma and serum, 69 (Abs.)
area, effect of a single topical fluoride treatment, 71 (Abs.)
dietary, utilization of, in presence of calcium salts, 70 (Abs.)
distribution in enamel, relation to fluoride in drinking water, 318
incorporation into enamel, 26 (Abs.)
in drinking water, relation to distribution in enamel, 318
in enamel, dentin and roots, 25 (Abs.)
ingestion, effects on organic matrix of teeth of growing pigs, 264
lack of effect on urinary calculi formation, 69 (Abs.)
levels, effect on caries in salivariadenectomized rats, 170
urinary, children and adults, 601
metabolism, effect of previous exposure to fluorine and age, 72 (Abs.)
and nonfluoride containing tin salts, effect on caries in children, 422
and phosphate exchange in mineralized tissues, mechanisms, 70 (Abs.)
placental transfer in rat, 326
radioactive, and other ions, rate of uptake and release by the hard dental tissues, 749 (Abs.)
half-life, 69 (Abs.)
removal, from solutions of sodium and stannous fluoride by powdered enamel, 748 (Abs.)
retention, and role of ascorbic acid in, 72 (Abs.)
salivary, effects on enamel solubility, 58 (Abs.)
topical application, effect of a single treatment in a fluoride area, 71 (Abs.)
new approach to, 71 (Abs.)
topically applied, potassium fluorostannite, three-year results, 71 (Abs.)
Fluoride—Cont'd
utilization of, effect of inorganic ions, in rats, 70 (Abs.)
Fluorides, distribution of tin and fluorides brushed on enamel, 24 (Abs.)
effect of sodium fluoride on extracts of fluoride-resistant *Lactobacillus casei*, 48 (Abs.)
natural fluoride content and solubility rate of surface and subsurface enamel, 254
potassium fluorostannite, topical, effect on caries in children, 417
reaction products of stannous fluoride and sodium fluoride with powdered dental enamel, identification, 58 (Abs.)
stannous fluoride and sodium fluoride, effects on enamel, electron microscopic observation, 638
-calcium pyrophosphate dentifrice, modified, effect on caries in children, 448
topical application, effect on caries in adult, 415
Fogels, H. R., 75 (Abs.), 82 (Abs.)
Food consumption, salivary flow and caries in rat, 407
Foodstuffs, adhesion to teeth, 72 (Abs.)
attempts to measure total acid potential, 73 (Abs.)
Forsaith, J., 32 (Abs.)
Forscher, B. K., 38 (Abs.)
Forziati, A. F., 86 (Abs.)
Fosdick, L. S., 23 (Abs.), 30 (2 Abs.), 51 (Abs.), 63 (Abs.), 73 (2 Abs.)
Frasher, L. A., 75 (Abs.)
Fremlin, J. H., 749 (Abs.)
French, W. C., 79 (Abs.)
Fullmer, H. M., 62 (Abs.)
Fusospirochetal lesions, experimental, in guinea pigs, localization of microorganisms, 10 (Abs.)

Gardner, A. F., 492
Gardner, D. E., 25 (Abs.), 254, 318, 748
Garn, S. M., 561
Gdalia, I., 601
Geiger, J. F., 8 (Abs.)
Geller, J. H., 26 (Abs.), 75 (Abs.), 107, 114, 276, 306, 453
Geminate composite odontome, inheritance of, in dog—pilot experiments, 755 (Abs.)
Germ-free animals, guinea pigs, 11 (Abs.)
Gerontology, comparative, periodontium of rat and hamster, 62 (Abs.)
Gerstner, R., 35 (Abs.), 37 (Abs.), 384
Gibilisco, J. A., 13 (Abs.)
Gilkerson, S. W., 26 (Abs.)
Gillis, R. E., 48 (Abs.)
Gillooly, C. V., 35 (Abs.)

Gilmore, H. W., 92 (Abs.), 816
Gilmore, R. W., 301
Gilmour, M. N., 9 (Abs.), 10 (Abs.)
Gingiva, attached, histochemical study in pregnancy, 64 (Abs.), 1115
fixed human, effect of enzymes, 63 (Abs.)
Gingival epithelium, development in golden hamster, 752 (Abs.)
hyperplasia, study of effect of anticonvulsant drugs on epileptic and mentally retarded patients, 64 (Abs.)
infections, experimental, biochemical and metabolic determinants, 62 (Abs.)
sulcus, healthy, presence of bacteria in, 288
tissues, human, availability of bacterial growth factors in, 427
Gingivitis and hyaluronidase activity of saliva, 114
Gish, C. W., 71 (2 Abs.), 417
Glasstone, S., 738
Glickman, I., 64 (Abs.), 119, 1115
Globulin, human, growth inhibiting activity of oral *Lactobacillus acidophilus*, 1167 (Annot.)
Glucose, effect of different concentrations on pH of plaque in vivo, 756 (Abs.)
Glycolysis, chemical inhibition in salivary sediment, film thickness as limiting factor, 798
and growth of oral acidogens, effect of bivalent trace minerals, 1149
inhibition, influence of film thickness as resistance to, 74 (Abs.)
Glyoxylic reactant in saliva, 788
Gochman, N., 30 (Abs.)
Going, R. E., 17 (Abs.)
Gold castings, micro studies, 95 (Abs.)
some effects of cavity roughness on adaptation of, 95 (Abs.)
Goldhaber, P., 18 (Abs.), 38 (Abs.)
Goodale, W. G., 43 (Abs.)
Goodlin, S. D., 31 (Abs.)
Gore, L. F., 754 (Abs.)
Gores, R. J., 21 (Abs.)
Gorlin, R. J., 19 (Abs.), 22 (Abs.), 64 (Abs.), 1141
Grabenstetter, R. J., 1060
Grafts, autogenous bone in treatment of intrabony periodontal pockets, 46 (Abs.)
heterologous anorganic bone response of oral tissues to, 44 (Abs.), 45 (Abs.)
Grainger, R. M., 853
Grand, N. G., 56 (Abs.)
Gray, J. A., 57 (Abs.), 638
Green, G. H., 43 (Abs.), 50 (Abs.)
Green, L. B., 175
Greulich, R. C., 5 (Abs.)
Grimes, C. U., 27 (Abs.)
Grisamore, T. L., 1167 (Annot.)
Grøn, P., 14 (Abs.)
Grossman, L. I., 67 (Abs.)
Ground substance, depolymerization of, in experimental infections, 49 (Abs.)
Growth abnormalities, effect of hypervitaminosis A and anomalous oculodentofacial pattern, 8 (Abs.)
and development of jaws and teeth, some effects of vitamin A, 752 (Abs.)
differential, of deciduous incisors, 760 (Abs.)
and enzymatic activities of oral "actinomyces," 9 (Abs.)
factors, bacterial, availability in human gingival tissues, 427
streptococcal, availability of, in human saliva, 527
hormone, and submaxillary salivary gland of rat, 56 (Abs.)
reactions of various rat epithelia to local injections, 37 (Abs.)
Guinea pigs, effect of vitamin C deficiency on condylar osteoclasts, 80 (Abs.)
experimental fusospirochetal infection, 10 (Abs.)
germ-free, infectivity of oral spirochetes for cortisone-treated, 11 (Abs.)
Gupta, O. P., 77 (Abs.)
Gypsum investments, dimensional change in wax patterns during setting, 351
materials, further evidence on hygroscopic expansion of, 95 (Abs.)

Haack, D. C., 136
Haldi, J., 33 (2 Abs.), 66 (Abs.)
Haley, M. I., 719
Hall, D. M., 243
Hall, H. D., 912
Hampp, E. G., 11 (2 Abs.)
Hamster, comparative gerontology of periodontium, 62 (Abs.)
development of gingival epithelium, 752 (Abs.)
dietary supplements and caries, 35 (Abs.)
effect of hyaluronidase on hemolytic staphylococcus abscesses, 49 (Abs.)
sialoadenectomy on periodontal tissue, 77 (Abs.)
topical application of carcinogens on palatal mucosa, 19 (Abs.)
frequency distribution of lactobacilli, 50 (Abs.)
homogenous transplantation of second molars from young to adult animals, 36 (Abs.)
influence of nongenetic factors in transmission of caries, 32 (Abs.)
Phenergan hydrochloride and extraction wound healing, 39 (Abs.)
postoperative evaluation of survival of second molars, 37 (Abs.)

Hamster—Cont'd
replantation of molar teeth, 36 (Abs.)
technic for root canal experiments in molar teeth, 40 (Abs.)
Handpieces, dental, measurement of torque-speed characteristics, 92 (Abs.)
HANOK, A., 631
HAPPLE, J. D., 79 (Abs.)
HARDINGE, M. G., 865, 874
HARDWICK, J. L., 749 (Abs.)
HARRIS, N. O., 47 (Abs.)
HARRIS, R. S., 35 (Abs.)
HARTLES, R. L., 755 (Abs.)
HARTLEY, J. L., 93 (Abs.), 94 (Abs.)
HATTON, M. E., 853
HAWKINS, W. L., 30 (Abs.), 688
HAYES, R. L., 938
HAYWARD, J. R., 36 (2 Abs.), 46 (Abs.)
Healing, artificial pockets in dogs, 46 (Abs.)
wound, effects of beta-aminopropionitrile, 38 (Abs.)
Phenergan hydrochloride and extraction in the hamster, 39 (Abs.)
HEIDENSLEBEN, L., 788
HEIN, J. W., 34 (Abs.), 75 (Abs.)
HENDERSHOT, L. C., 32 (Abs.)
HENRIQUES, B. L., 28 (Abs.)
Heterotopic osteogenic effect of calcium hydroxide in connective tissue, 47 (Abs.)
HEWES, C. G., 32 (Abs.)
High speed rotary operative dental procedure, biology of. I. tooth volume and pulpal pathology, 67 (Abs.)
HILGER, E. C., 31 (Abs.)
HINDS, E. C., 45 (Abs.)
Histochemical study of attached gingiva in pregnancy, 1115
HITCHIN, A. D., 755 (Abs.)
HOBBS, G., 754 (Abs.)
HODGE, H. C., 748 (Abs.)
HOEK, R. B., 36 (2 Abs.)
HOERMAN, K. C., 906
HOLDER, T. D., 162
HOLLOWAY, P. J., 752 (Abs.)
HOLTON, P. J., 41 (Abs.)
HOPPE, J. O., 206
HOPPER, F. E., 759 (Abs.)
HOPPERT, C. A., 54 (Abs.), 55 (Abs.), 824
Hormonal control of submaxillary and pancreatic proteolytic activity, 485
HOUSTON, M., 20 (Abs.)
HOWARD, L. V., 669
HOWELL, C. L., 71 (2 Abs.), 417
HUDSON, D. C., 90 (Abs.)
HUGHSTON, H. H., 623
HÜRZELER, B., 44 (Abs.), 1035
HUNT, H. R., 54 (Abs.), 55 (Abs.), 824
HUNTER, H. A., 10 (Abs.)
HUNTER, P., 10 (Abs.)
HUNTER, S., 15 (Abs.)
Hyaluronidase activity of saliva. I. determination of characteristics, 107
II. relationship to caries experience, gingivitis, and oral hygiene, 114
VI. effect of antibiotic therapy, 453
experience and caries susceptibility tests, 306
effect on hemolytic staphylococcus abscesses in hamsters, 49 (Abs.)
Hydrocolloid impression materials, effect of residual stress on deformation, 92 (Abs.), 816
Hydrocortisone and polyantibiotics, effect on rat molar pulp, 886
Hygroscopic expansion dental gypsum materials, further evidence, 95 (Abs.)

Implants, bovine and anorganic bone in human being, histologic comparison, 45 (Abs.)
Impression materials, elastic, determination of shrinkage, 92 (Abs.)
hydrocolloid, effect of residual stress on deformation, 92 (Abs.) 816
silicone rubber as, 93 (Abs.)
chemical and physical studies, 93 (Abs.)
Infected pulpless teeth, bacteriologic status of periapical tissue, 67 (Abs.)
Inflammation, acute, effect of age on, 38 (Abs.)
INGERSOLL, C. E., 95 (Abs.)
Inlay pattern waxes, indirect, evaluation of their manipulation, 95 (Abs.)
Inorganic beef bone matrix, use of, in oral surgery, 46 (Abs.)
Intelligence quotient, in children with congenital cleft palate, 79 (Abs.)
Intercast dimensions, changes in, produced by mounting procedures, 94 (Abs.)
Intermediate bifurcational ridge, 162
International Association for Dental Research, announcement of 1959 General Meeting, 765, 990
preprinted abstracts of papers presented at 36th General Meeting, March, 1958, 4
proceedings of the British division, 748
of Northwest division, 179
of the Thirty-Sixth General Meeting, March, 1958, 951
scientific program of the Chicago section, 762
Intraoral cytology, diagnostic value, 195
IRVING, J. T., 732
Isotopes, *see* Radioactive isotopes
ISAAC, S., 254, 318

JACKSON, F. L., 1060
JACKSON, S. W., 51(Abs.)
JEFFAY, H., 6 (Abs.)

JENKINS, G. N., 759 (Abs.)
JOHANSEN, E., 33 (Abs.), 34 (Abs.), 50 (Abs.)
JOLY, O., 37 (Abs.), 280
JONES, M., 8 (Abs.), 697
JORDAN, H. V., 52 (Abs.)
JOSEPH, N. R., 39 (Abs.), 40 (Abs.)
JUMP, E. B., 162

KEAN, M. R., 15 (Abs.)
KEATING, N., 35 (Abs.)
KENNEDY, J. J., 94 (Abs.)
KENNY, A. D., 432
KERR, A. C., 756 (Abs.)
KESEL, R. G., 50 (Abs.)
KEYES, P. H., 32 (Abs.), 1077, 1088
KIMBER, W. J., 51 (Abs.)
KIMMEL, M. D., 87 (Abs.)
KING, C. T. G., 7 (Abs.)
KING, N., 31 (Abs.)
KING, W. J., 74 (Abs.)
KIRYATI, A. A., 886
KLAPPER, C. E., 53 (Abs.)
KLEINBERG, I., 756 (Abs.)
KLINGSBERG, J., 62 (Abs.)
KNAPP, D. E., 7 (Abs.), 880
KNIGHTON, H. T., 67 (Abs.)
KNOEDLER, D., 614
KOSKI, K., 561
KOSLOW, A. J., 81 (Abs.)
KOULOURIDES, T., 57 (Abs.)
KOZAM, G., 605
KRAMER, I. R. H., 758 (Abs.)
KRESHOVER, S. J., 19 (Abs.)
KRIKOS, G. A., 38 (Abs.)
KUMAMOTO, Y., 147
KUMPULA, J. W., 92 (Abs.)
KUNKLE, M. F., 51 (Abs.), 780
KURTZ, H. M., 427, 527
KVAM, T., 532, 540

Lactobacilli counts after elimination of carious lesions, 50 (Abs.)
LANGER, H. G., 58 (Abs.)
LARSON, R. H., 52 (Abs.)
LASATER, R. L., 91 (Abs.)
LASKIN, D. M., 39 (Abs.), 40 (Abs.)
Lathyrism, experimental, masticatory apparatus in rat, 492
LAW, M. L., 33 (Abs.)
LAWRENCE, A. H., 41 (Abs.)
LAWTON, F. E., 755 (Abs.)
LEACH, S. A., 748 (Abs.)
LEBLOND, C. P., 147
LEFKOWITZ, W., 1100
Leukocytes, studies on disintegration of, in saliva on incubation, 756 (Abs.)
LEUNG, S. W., 77 (Abs.), 78 (Abs.)
Levo- and dextro-nordefrin, some pharmacologic properties, 206
LEVY, B. M., 950 (Annot.)
LEWIS, A. B., 561
Ligation atrophy, submaxillary gland of rat, 54 (Abs.)
Limulus cartilage, comparative studies of calcified and skeletal tissues, 4 (Abs.)
LINDENMEYER, R. S., 91 (Abs.)
LISANTI, V. F., 63 (Abs.)
LITTLE, K., 749 (Abs.)
LITTLE, M. F., 26 (Abs.), 991
Longitudinal study of tooth calcification and root resorption, 4 (Abs.)
LOSEE, F. L., 23 (Abs.), 44 (Abs.), 45 (Abs.)
LOTZ, W. E., 264
LUDUENA, F. P., 206
LUX, V. J., 28 (Abs.)
LYON, H. W., 44 (Abs.), 45 (Abs.)

Macaca rhesus, 759 (Abs.)
MCCALL, C. M., 14 (Abs.)
MCCANN, H. G., 70 (Abs.)
MCCONNELL, D., 26 (Abs.)
MCDONALD, R. E., 76 (Abs.)
MCEWEN, R. A., 94 (Abs.)
MCGREGOR, R. R., 93 (Abs.)
MCHUGH, W. D., 752 (Abs.)
MACINTOSH, R. B., 42 (Abs.)
MACKENZIE, R., 81 (Abs.)
MACLAUGHLIN, J. A., 75 (Abs.)
MCMILLAN, L., 51 (Abs.)
MADLENER, E. M., 10 (Abs.), 12 (Abs.)
MAHLER, D. B., 29 (Abs.), 95 (Abs.), 516
Mandible, tumors of, malignant lymphoma, 21 (Abs.)
Mandibular condyle, osteoclast counts on vitamin C deficient guinea pigs, 80 (Abs.)
movement, in children, serial cephalograms, 15 (Abs.)
MANERI, R. R., 93 (Abs.)
MANHOLD, J. H., JR., 77 (Abs.), 330
MANLEY, E. B., 751 (Abs.)
MANLY, R. S., 74 (Abs.), 798
MANTEL, N., 938
MARCHESSEN, H., 34 (Abs.)
MARFINO, N. R., 46 (Abs.)
MARSLAND, E. A., 750 (2 Abs.).
MASHIMO, P. A., 28 (Abs.)
MASSLER, M., 17 (Abs.), 48 (Abs.), 229
Mast cells, rat, variations in cytochemistry of, 758 (Abs.)
Masticatory apparatus in albino rat in experimental lathyrism, 492
stresses, normal and modified occlusions, 312
MATT, M. M., 27 (Abs.)
MATTHEWS, J. L., 80 (Abs.)
MAU, L. M., 906
Maxilla, rat, effect of stress on maxillary suture, 80 (Abs.)
tumors of, malignant lymphoma, 21 (Abs.)
MAZZARELLA, M. A., 31 (Abs.)
MELLANBY, M., 752 (2 Abs.)
MELVILLE, T. H., 754 (Abs.)

Mercury analysis by fluorescent x-ray spectroscopy, 359
MERGENHAGEN, S. E., 48 (Abs.)
Metabolic significance of collagen in tooth structure, 276
Methyl-methacrylate co-polymers, vapor phase chromatography analysis, 86 (Abs.)
MEYER, J., 54 (Abs.), 485
MEYER, R., 30 (Abs.)
MEYERS, C. E., 175
Microbial decomposition of enamel and dentin in vitro, 48 (Abs.)
Microcinematography of living dental pulp, 68 (2 Abs.)
Microhardness of enamel and dentin, 91 (Abs.), 661
tests, semiquantitative studies of in vitro caries, 301
Microorganisms, bacteriophage, specific for *Lactobacillus casei* from human oral material, 175
Clostridium histolyticum, action of culture filtrates on decalcified bone and dentin, 754 (Abs.)
filamentous organism, growth characteristics, 10 (Abs.)
fusobacteria, some studies on nutritional requirements of, 18 (Abs.)
lactobacilli, frequency distribution of, from the hamster, 50 (Abs.)
Lactobacillus acidophilus, growth inhibiting activity of human globulin, 1167 (Annot.)
time-function studies of pH and uptake of potassium, sodium, and phosphorus during the growth cycle, 47 (Abs.)
casei, fluoride resistant, effect of sodium fluoride on extracts, 48 (Abs.)
isolation of bacteriophage specific for, 175
Leptothrix racemosa, microscopic studies, 9 (Abs.)
localization of, in experimental fusospirochetal lesions in guinea pigs, 10 (Abs.)
oral acidogens, 1149
"actinomyces," growth and enzymatic activities, 9 (Abs.)
spirillum, isolation and characterization, 12 (Abs.)
spirochetes, in vitro effects of antibiotics, 11 (Abs.)
protozoa, habitat and related conditions, 12 (Abs.)
human mouth, studies on, 949 (Annot.)
salivary gland virus, necrotizing factor isolated from mouse salivary glands exposed to, 56 (Abs.)
Serratia marcescens, utilization of, in study of anachoresis in mice, 42 (Abs.)
staphylococcus, hemolytic effect of hyaluronidase on abscesses in hamsters, 49 (Abs.)
Microorganisms—Cont'd
streptococci, beta hemolytic, inhibitory effect of saliva, 654
in vitro inhibition by saliva, 50 (Abs.)
Microradiography, high resolution, dental organic matrices studies, 5 (Abs.)
MIGLANI, D. C., 61 (Abs.)
Migration, mesial, teeth of *Macaca rhesus*, 759 (Abs.)
MILES, A. E. W., 757 (Abs.)
MILLER, G. E., 95 (Abs.)
Mineralization, pattern of, in human enamel, 750 (2 Abs.)
Mineralized tissues, mechanisms of phosphate and fluoride exchange in, 70 (Abs.)
MINK, J. R., 12 (Abs.)
MITCHELL, D. F., 47 (Abs.), 1157
MIYAHARA, R., 39 (Abs.)
Mobility, tooth, development of instruments to measure, 64 (Abs.)
MOFFITT, R. E., 95 (Abs.)
MOHAMMED, C. I., 6 (Abs.)
Moisture, content of calcified tooth tissues, 581
MOLNAR, E. J., 92 (Abs.)
MOORE, E. E., 80 (Abs.)
MOORREES, C. F. A., 15 (Abs.)
MORRIS, A. L., 17 (Abs.)
MORRIS, I., 755 (Abs.)
MOSHONAS, M. F., 547
Mouse, caries and thyroid activity, 451
experimental carcinoma of tongue, 950 (Annot.)
histochemical study of salivary glands, normal and cortisone injected, 119
histologic changes in gonads following parotin injection, 41 (Abs.)
necrotizing factor isolated from salivary glands exposed to salivary gland virus, 56 (Abs.)
observations on some genetic characteristics of the periodontium, 61 (Abs.)
salivary gland tumor growth characteristics, host response, 41 (Abs.)
study of anachoresis utilizing *Serratia marcescens*, 42 (Abs.)
MOYERS, R. E., 15 (Abs.)
Mucosa, oral, total pattern of innervation, 753 (Abs.)
MUHLER, J. C., 16 (Abs.), 23 (Abs.), 53 (2 Abs.), 54 (Abs.), 56 (Abs.), 58 (Abs.), 70 (2 Abs.), 71 (3 Abs.), 72 (2 Abs.), 170, 326, 407, 410, 412, 415, 417, 419, 422, 448, 451, 649, 848, 860, 1069
MUMFORD, G., 351
MUNSON, P. L., 55 (Abs.)
MYERS, G. E., 93 (Abs.)
MYERS, H. I., 35 (Abs.), 37 (Abs.), 39 (Abs.), 47 (Abs.), 49 (Abs.), 80 (Abs.)
MYERS, H. M., 25 (Abs.)

NASH, L. L., 214
NEALON, F. H., 89 (Abs.)
NEBERGALL, W. H., 58 (Abs.)
Nerve sclerosants, histological evaluation, 81 (Abs.)
Neural impulse transmission in human teeth, 14 (Abs.)
NEVIN, T., 427, 527
NEWBRUN, E., 56 (Abs.), 60 (Abs.)
NEWCOMER, C. E., 43 (Abs.)
New material for somato-prosthesis, 948 (Annot.)
NEWTON, W. L., 11 (Abs.)
NIEDERMEIER, W., 1149
NIELSEN, A. G., 91 (Abs.), 94 (Abs.)
NIIZIMA, M., 767
Nitrogen content of dental pulp, further studies, 214
NIZEL, A. E., 35 (Abs.)
NOLTE, W. A., 9 (Abs.)
Northwest Division IADR, proceedings, 179
NYLEN, M. U., 22 (Abs.)

O'BRIEN, C., 467
Occlusion, normal and modified, masticatory stresses, 312
Octocalcium phosphate and whitlockite, formation and stability, 78 (Abs.)
O'DAY, P., 719
Odontome, geminated, composite, inheritance of, in dog—pilot experiments, 755 (Abs.)
O-Ethoxybenzoic acid, reaction of metal oxides with, and other chelating agents, 547
OGLESBY, P. L., 89 (Abs.)
OMATA, R. R., 10 (Abs.)
Oral mucosa, ultrastructural observations, 18 (Abs.)
surgery, tranquilizing drugs, 14 (Abs.)
use of inorganic beef bone matrix in, 46 (Abs.)
ORBAN, B. J., 46 (Abs.)
ORBISON, J. L., 38 (Abs.)
Organic matrix of teeth of growing pigs, effects of fluoride ingestion, 264
Orthodontics and supra-alveolar fibers, 47 (Abs.)
O'SHAUGHNESSY, P. E., 16 (Abs.)
Osteoclasts, effects of vitamin C deficiency, 80 (Abs.)
Osteogenic potential of calcium hydroxide and other material in wounds, 1157
Oxygen quotients, comparison of bovine, sheep and human dental pulp, 65 (Abs.)
OYEN, I. H., 206

Palate contour, as a limiting factor in intraoral x-ray technic, 14 (Abs.)
PANUSKA, H. J., 64 (Abs.)
Parabiosis in rats, 912
PARFITT, G., 64 (Abs.)
Parotid, *see* Salivary glands
Parotin, histologic changes in gonads of mice following injection, 41 (Abs.)
PEPPER, M. B., 623
Peptides, effect on acid formation in sugar-saliva mixtures, 73 (Abs.)
Periodontal condition, correlation of blood constituents, 63 (Abs.)
disease and bacterial enzymes, 100
and sources of hydrolytic enzymes, 63 (Abs.)
tissues, autogenous bone grafts in treatment of intrabony periodontal pockets, 46 (Abs.)
comparative gerontology of, in rat and hamster, 62 (Abs.)
effect of sialoadenectomy on, in hamster, 77 (Abs.)
vitamin A deficiency on periodontal structures of rat molars, 61 (Abs.)
electron microscopic study of particulates in periodontal exudate, 51 (Abs.)
experimental gingival infections, biochemical and metabolic determinants, 62 (Abs.)
healing of artificial pockets in dogs, 46 (Abs.)
new stain for connective tissues of, 62 (Abs.)
observations on some genetic characteristics of the periodontium in mice, 61 (Abs.)
organization of the periodontal fibers of developing molars, 61 (Abs.)
reattachment following trauma and infection, 759 (Abs.)
supra-alveolar fibers and orthodontic treatment, 47 (Abs.)
Periodontium, *see* Periodontal tissues
PERKINS, R. R., 92 (Abs.), 94 (Abs.)
PERSON, P., 4 (Abs.), 62 (Abs.), 292
PETROPOULOS, G. J., 13 (Abs.)
PEYTON, F. A., 91 (Abs.), 661, 710
PHATAK, N. M., 24 (Abs.)
Phenergan hydrochloride and extraction wound healing in hamster, 39 (Abs.)
PHILLIPS, R. W., 88 (Abs.), 92 (Abs.), 351, 811, 816, 837
Phosphate and fluoride exchange in mineralized tissues, mechanisms, 70 (Abs.)
supplements to diet and hamster caries, 35 (Abs.)

Photographic technics for fluorescent and white light observations of the same tissue section, 20 (Abs.)
Photomicrographic technic using broad spectrum light of ultrashort duration, 17 (Abs.)
PICTON, D. C. A., 312
PIGMAN, J., 301
PIGMAN, W., 30 (Abs.), 56 (Abs.), 301, 688
PILKEY, L., 79 (Abs.)
Pilocarpine, effect on caries in rat, 410
effect on submaxillary gland in rat, 649
Pima Indian children, emergence of permanent teeth, 1123
Placental transfer of fluoride in rat, 326
Plaque, effect of different concentrations of glucose on pH in vivo, 756 (Abs.)
effect of saliva on rate of acid formation, 73 (Abs.)
Platinic chloride, effect of on experimental dental caries, 34 (Abs.)
POHTO, M., 68 (2 Abs.)
POLACHECK, D. L., 561
Polishing agents, study of surface reaction of enamel, 91 (Abs.)
POLLAK, V. E., 40 (Abs.)
Polycythemia, cobalt-induced, 1141
Polymethyl methacrylate, rate and degree of polymerization of, effect of varying peroxide and polymer, 86 (Abs.)
Polysaccharides of tissues, cation binding in vitro, 42 (Abs.)
Polyvinyl resin sponge, study of, in jaws of dogs, 45 (Abs.)
POOL, M. L., 15 (Abs.), 920
POOLE, W. L., 52 (Abs.)
Potassium fluorostannite, topically applied, three-year results, 71 (Abs.)
POWERS, C. M., 86 (Abs.), 87 (Abs.)
Pregnancy, histochemical study of attached gingiva, 64 (Abs.), 1115
Preservatives, surface effects, 24 (Abs.)
Presidential inaugural address, 373, 951
Pressure injection of whole blood into intermedullary spaces of dog mandibles, histologic changes produced by, 41 (Abs.)
Protective factors, further observations on occurrence in unrefined foods, 759
Protein depletion, response of adult rat oral tissues, 292
Proteolytic activity of submaxillary gland and pancreas, hormonal control, 485
Psychologic predisposition in cavity formation, 330
PUGH, D. G., 21 (Abs.)
Pulp, bovine, qualitative chromatographic analysis of amino acids, 65 (Abs.)
comparison of oxygen quotients of bovine, sheep, and human, 65 (Abs.)
Pulp—Cont'd
differential staining of undecalcified sections, 751
dog, early changes in, following full crown preparation, 220
exposed, influences affecting repair, 678
further studies on nitrogen content of, 214
healing following capping in human sound and carious teeth, 66 (Abs.)
microcinematography of living, 68 (Abs.)
rat and rabbit incisor, respiration, 605
molar, bacterial localization in, following bacteremias, 67 (Abs.)
effect of hydrocortisone and polyantibiotics, 886
unexposed, effect of calcium hydroxide, 758
Pulpal reactions, human, following 20,000 and 150,000 r.p.m. cavity preparations, 68 (Abs.)
Pulpotomy, experimental molar in rat, 229
Pyridoxine and injected carbohydrate, effect on caries, dentinal circulation, 874

QUIGLEY, G. A., 34 (Abs.)

RADCLIFF, R. E., 70 (Abs.)
RADDEN, B. G., 758 (Abs.)
Radiations and radiography, cephalographic studies of mandibular movement in children, 15 (Abs.)
irradiation from dental unit, effect on rat, 16 (Abs.)
palate contour as a limiting factor in intraoral x-ray technic, 14 (Abs.)
production of clinical roentgenograms by compact radioactive x-ray and gamma-ray sources, 920
quantitative assessment of radiodensity and birefringence in incipient carious lesions, 60 (Abs.)
scintillation spectra from dental x-ray machines, 15 (Abs.)
x-radiation, prenatal effects on bones, teeth, and periodontium, 16 (Abs.)
x-ray spectroscopy, fluorescent, mercury analysis by, 359
RADIKE, A. W., 1060
Radioactive isotopes, C^{14} visualization in tooth matrix after administration of labeled hexoses, 147
fluoride, half-life, 69 (Abs.)
fluorides, radioactive, and other ions, rate of uptake and release by the hard dental tissues, 749
fluorine-18, autoradiographs of solder joints, 96 (Abs.)
I^{131} penetration into fillings, 17 (Abs.)
labeled hexoses, visualization of C^{14} in tooth matrix after administration, 147
radiotracer evaluation in bones and teeth, quantitative, topographic sample, 458

Radioactive—Cont'd
teeth, utilization of, measurement of dentifrice abrasion, 1060
Rapp, R., 14 (Abs.)
Rat, albino, masticatory apparatus in experimental lathyrism, 492
alteration of blood flow to teeth, 1069
bacterial localization in molar pulp following bacteremias, 67 (Abs.)
caries, effect of diet of barley and fish, 419
of selenium salts, 35 (Abs.)
of trace element sea salt mixture, 33 (Abs.)
experimental, and $CaHPO_4$ dietary supplement, 34 (Abs.)
high sucrose diet during calcification of teeth, 53 (Abs.)
influence of alloxan injections on development, 755 (Abs.)
initiation and promotion factors in, 31 (Abs.)
occlusal, effect of sugar in various forms, 570
caries-resistant and caries-susceptible, Harvard and Hunt-Hoppert, salivary protease activity, 930
Hunt-Hoppert, caries incidence and parotid extirpation and duct excision, 722
salivariadenectomized, studies, 824
salivary gland extirpation and duct ligation, 55 (Abs.)
studies on Hunt-Hoppert, salivary gland extirpation, 54 (Abs.)
cariogenic effect of adding sugar cane syrup to diet, 758 (Abs.)
comparative gerontology of periodontium, 62 (Abs.)
effect of diet on bones and teeth, 6 (Abs.)
inorganic ions on utilization of fluoride, 70 (Abs.)
local injection of somatotrophic hormones in small doses into oral mucosa, 280
metal salts of EDTA on experimental caries, 32 (Abs.)
pilocarpine on dental caries, 410
on submaxillary gland, 649
vitamin A deficiency in periodontal structures of molars, 61 (Abs.)
eruptive mechanism and movement in first molar, 467
experimental molar pulpotomies, 229
oral ulceration, 38 (Abs.)
fluoride, placental transfer, 326
food consumption, salivary flow and caries, 407
growth abnormalities, 8 (Abs.)
heterotopic osteogenic effect of calcium hydroxide in connective tissue, 47 (Abs.)
Hunt-Hoppert, *see* Rat, caries-resistant
hypervitaminosis A, 8 (Abs.)
ia, calcium and phosphorus metabolism, 432
influence of sialoadenectomy on food and water consumption, 805
Rat—Cont'd
mast cells, variations in cytochemistry, 758 (Abs.)
maxillary suture and controlled mechanical stress, 80 (Abs.)
molar teeth, effect of acid drinks, 752 (Abs.)
parabiosis in, 912
pulp, effect of hydrocortisone and polyantibiotics, 886
and rabbit incisor pulp, respiration, 605
radiation effects, 16 (Abs.)
relation of feeding regimens to caries, 52 (Abs.)
response of adult oral tissue to protein depletion, 292
salivariadenectomized, effect of fluoride levels on caries, 170
salivary gland function. I. flow, viscosity, pH, 848
submaxillary gland, ligation atrophy, 54 (Abs.)
suckling, intraperitoneal carbohydrate injections and caries, 719
vitamin E depleted, curative effect of alpha-tocopherol and protein, 732
white, systemic etiological factors of caries, 32 (Abs.)
Rattner, L. J., 79 (Abs.)
Reattachment of periodontal tissues following trauma and infection, 759 (Abs.)
Reducing power of saliva and its component secretions, 688
Relaxing drug for operative dentistry, clinical dental trial. II, 444
Reliability of measurements from cephalograms at Burlington Orthodontic Centre, 853
Remo, J. W., 12 (Abs.), 949 (Annot.)
Replantation of molar teeth in the Syrian hamster, 36 (Abs.)
Resnick, J. B., 722, 930
Respiration of rat and rabbit incisor pulp, 605
of salivary microbial flora, influence of caries activity, 43
Restorative materials, effect on dentin solubility, 811
response to thermal variations, 89 (Abs.)
Richards, J. R., 91 (Abs.)
Richardson, R. L., 8 (Abs.), 697
Richter, V. J., 74 (Abs.)
Rigas, D. F., 91 (Abs.)
Robinson, J. E., 948 (Annot.)
Rogosa, M., 50 (Abs.)
Root canal cultures, occurrence of branching bacteria, 754 (Abs.)
resorption, longitudinal study of tooth calcification and, 4 (Abs.)
Rosen, S., 54 (Abs.), 55 (Abs.), 824
Rosenthal, L., 288
Rosevear, F. B., 638
Rovelstad, G. H., 26 (2 Abs.), 75 (Abs.), 107, 114, 306, 453

ROWBOTHAM, T. C., 753 (Abs.)
ROWE, N., 19 (Abs.)
ROWLES, S. L., 749 (Abs.)
ROWLEY, J., 26 (Abs.)
Rustless alloys, plated, solder joints, 368
RYGE, G., 88 (2 Abs.), 96 (Abs.), 359

SALEM, Massachusetts, dental evaluation of 200 public school children, 76 (Abs.)
Saliva, availability of streptococcal growth factors, 527
chemical fractionation, 27 (Abs.)
and component secretions, reducing power of, 688
effect on rate of acid formation in dental plaques, 73 (Abs.)
electrochemistry, 27 (Abs.)
electrophoresis, 26 (Abs.)
flow rate and lactate from parotid, 29 (Abs.)
human, bacteriologic census, 8 (Abs.), 697
whole stimulated, sodium and potassium levels, 391
hyaluronidase activity, 107, 114
immunochemical studies, 28 (Abs.)
incubated, effects of glucose, lactate and pH on certain bacterial and chemical changes, 51 (Abs.)
influence of caries activity on respiration of microbial flora, 43 (Abs.)
in vitro chemical and bacterial changes, 780
inhibition of beta hemolytic streptococci, 50 (Abs.)
inhibitory effect on beta hemolytic streptococci, 654
ionic constituents, metallic elements, 28 (Abs.)
lactate from parotid, 29 (Abs.)
parotid, effect of ingested carbohydrates on concentrations of amylase, orthophosphates and carbon dioxide, 755 (Abs.)
electrolyte secretion, governing mechanisms, 29 (Abs.)
manometric studies of micro-organisms in, 51 (Abs.)
pH, titratable alkalinity, rate of flow and caries activity, 906
production of ammonia and volatile amines, 30 (Abs.)
reducing power, 30 (Abs.)
studies on disintegration of leukocytes on incubation, 756 (Abs.)
submaxillary and parotid, electrolytic concentrations, 28 (Abs.)
the glyoxylic reactant, 788
Salivariadenectomy, *see* Salivary gland extirpation
Saliva-sugar mixtures, effect of peptides on acid formation, 73 (Abs.)
Salivary flow, food consumption and caries in rat, 407
Salivary glands, carcinogenesis in duct-ligated submaxillary, 19 (Abs.)
effect of carbohydrates on function, 53 (Abs.)
extirpation and duct ligation, effect on caries, 54 (Abs.)
in caries-resistant and caries-susceptible rats, 55 (Abs.)
effect of fluoride levels on caries, 170
effects on periodontal tissues of the hamster, 77 (Abs.)
in Hunt-Hoppert rats, 54 (Abs.), 824
influence on food and water consumption in rat, 805
function in normal and duct-ligated rat. I. flow, viscosity, and pH, 848
histochemical study in normal and cortisone injected mice, 119
human submaxillary and parotid, effect of secretion of on some bacteria, 756 (Abs.)
metallic elements in parotid secretion, 28
mouse, necrotizing factor from exposure to salivary gland virus, 56 (Abs.)
parotid, extirpation and duct excision, caries incidence in Hunt-Hoppert rats, 722
flow rate and lactate, 29 (Abs.)
structure and function in rat. II. alteration of blood flow to teeth, 1069
submaxillary, bovine, calculus formation from a substrate containing an extract of, 78 (Abs.)
characteristics of extract used for calculus formation, 77 (Abs.)
effect of growth hormone and thyroxine, 56 (Abs.)
thyroxine on weight, 55 (Abs.)
and pancreatic proteolytic activity and hormonal control, 485
rat, effect of pilocarpine, 649
ligation atrophy, 54 (Abs.)
tumor, mouse, growth characteristics, host response, 41 (Abs.)
protease activity in Harvard and Hunt-Hoppert rats, 930
secretion, parotid, chloride levels, 902
sediment, amino acid decarboxylases in, 30 (Abs.)
chemical inhibition of glycolysis in, film thickness as limiting factor, 798
microscopic study of, 26 (Abs.)
sodium, potassium and chloride levels and dental caries experience, 401
SALLEY, J. J., 19 (Abs.)
SANDLER, H. C., 20 (Abs.)
SAVARA, B. S., 24 (Abs.)
SAVCHUCK, W. B., 5 (Abs.), 1164
SCHEININ, A., 68 (2 Abs.)
SCHERP, H. W., 48 (Abs.), 49 (Abs.)
SCHOUR, I., 432
SCHWARTZ, A., 722
SCHWEIZER, H. C., 638

Scintillation spectra from dental x-ray machines, 15 (Abs.)
Scofield, H. H., 7 (Abs.)
Scott, D. B., 22 (Abs.), 23 (Abs.), 759 (Abs.)
Scurvy, localization of vitamin C in dental tissues, 6 (Abs.)
Sebaceous glands, buccal, development and atrophy of, in man, 757 (Abs.)
Selenium salts, effect on caries production in rats, 35 (Abs.)
Seltzer, S., 220, 678
Sensitivity of human dentin, 669
Shade matching and color vision, defective, in dentistry, 760 (Abs.)
Shafer, W., 19 (Abs.), 54 (Abs.), 80 (Abs.), 848
Shankwalker, G. B., 1157
Shannon, I. L., 391, 401
Shapiro, M., 16 (Abs.)
Shaw, J. H., 33 (Abs.), 458, 722, 805, 912 930
Sheck, J., 69 (Abs.)
Shiere, F. R., 75 (Abs.), 82 (Abs.)
Shiota, T., 51 (Abs.), 780
Ship, I. L., 21 (Abs.)
Shklair, I., 29 (Abs.), 50 (Abs.), 73 (Abs.)
Shklar, G., 119
Sialoadenectomy, *see* Salivary glands, extirpation
Silica-resin, direct filling material, synthesis, 90 (Abs.)
Silicate cements, investigations to reduce solubility, 89 (Abs.)
standardization of colors, 89 (Abs.)
Silicone rubber as impression material, 93 (Abs.)
impression materials, clinical and physical studies, 93 (Abs.)
Silicones, surface effects, 24 (Abs.)
Silverman, S., Jr., 195
Singer, L., 69 (3 Abs.)
Skeletal development, effect of Ca reduction on, in *Echinarachnius parma* embryos, 6 (Abs.)
Skinner, E. W., 91 (2 Abs.)
Smith, F. A., 25 (Abs.), 254, 318, 748 (Abs.)
Smith, R. L., 187
Snyder, M. L., 179
Sobel, A. E., 631
Sodium and potassium levels of human whole stimulated saliva, 391
N-lauroyl sarcosinate dentifrice, effect on dental caries in children, 75 (Abs.)
N-palmitoyl sarcosinate, effect of temperature on powdered enamel solubility reduction, 74 (Abs.)
potassium and chloride levels of saliva and dental caries experience, 401
Sognnaes, R. F., 18 (Abs.), 373, 458
Solder joints, autoradiographs of, with fluorine 18, 96 (Abs.)
on plated rustless alloys, 368
Soldering dental bridge, method for evaluation of dimensional changes, 96 (Abs.)
Somato-prosthesis, new material for, 948 (Annot.)
Somatotropin, effect on temporomandibular joint, localized effects, 81 (Abs.)
Soni, N., 60 (Abs.)
Spangenberg, H. D., Jr., 15 (Abs.), 920
Spear, L. B., Jr., 70 (Abs.), 72 (Abs.)
Spies, T. D., 1149
Spinella, D. J., 65 (2 Abs.)
Sreebny, L. M., 54 (Abs.), 55 (Abs.), 485, 824
Stack, M. V., 760 (Abs.)
Stahl, S. S., 20 (Abs.), 37 (Abs.), 280
Standish, S. M., 19 (Abs.)
Stanford, J. W., 87 (Abs.)
Stanley, H. R., Jr., 66 (Abs.), 68 (Abs.)
Stanmeyer, W. R., 7 (Abs.), 614
Stasik, J. F., 31 (Abs.)
Steadman, L. T., 25 (Abs.)
Steelmon, G., 66 (Abs.)
Steg, R. F., 21 (Abs.)
Steinman, R. R., 32 (Abs.), 719, 865, 874
Stephan, R. M., 60 (Abs.)
Sterilization, dental instrument, use of ultrasonic energy, 43 (Abs.)
Stewart, D. J., 758 (Abs.)
Stewart, R. J. C., 752 (Abs.)
Stookey, G. K., 72 (Abs.)
Strain, W., 33 (Abs.)
Strassburger, J., 86 (Abs.)
Streptococcal growth factors, availability of, in saliva, 527
Strontium, distribution of in teeth from different geographical area, 25 (Abs.)
Structure, internal, technique for study of, in calcified tissues, 880
Submandibular gland, *see* Salivary glands
Sugar cane syrup, 758 (Abs.)
various forms, effect of, in occlusal caries in molars of rat, 570
Sullivan, R. P., 15 (Abs.)
Sundstrom, C., 35 (Abs.)
Superstine, M., 15 (Abs.)
Supporting Associates, 182, 568, 764, 988, 1168
Surveyor, electromechanical, for dental models, 90 (Abs.)
Swartz, M., 88 (Abs.), 92 (Abs.), 811, 816, 837
Swayne, P., 1100
Swerdlow, H., 68 (Abs.)
Symposium on modern methods of removal of tooth structure, 94 (Abs.)
Szmyd, L., 14 (Abs.)

Tachometer for evaluation of rotary cutting speeds, 91 (Abs.)
Takuma, S., 7 (Abs.)
Tanner, H. M., 94 (Abs.)
Tarnish of certain dental alloys, 837

TAYLOR, D. F., 92 (Abs.)
TAYLOR, G. W., 21 (Abs.)
TAYLOR, P. B., 94 (Abs.)
Teeth, carbonate content, 26 (Abs.)
effect of diet, 6 (Abs.)
roots, fluoride content, 25 (Abs.)
surface effects, 24 (Abs.)
Temporomandibular joint, effect of somatotropin, 81 (Abs.)
TEN CATE, A. R., 758 (Abs.)
Therapeutics, analgesic agents in, 13 (Abs.)
THOMPSON, H. E., 47 (Abs.)
THONARD, J. C., 49 (Abs.)
Thyroid activity and caries in mice, 451
Thyroxine, and submaxillary salivary gland of rat, 56 (Abs.)
effect on weight of submaxillary salivary glands, 55 (Abs.)
TIMBERLAKE, P., 56 (Abs.), 301
Tissue culture, dental papilla, 767
TOEPEL, W., 432
TONGE, C. H., 760 (Abs.)
TONZETICH, J., 74 (Abs.)
Tooth buds, normal development of in vitro cultures, 1100
calcification, longitudinal study of, and root resorption, 4 (Abs.)
germ and pulp development, in vitro studies, 384
germs, experimental studies on calcification of, in vitro, 738
preservation and storage of, 35 (Abs.)
Topographic sampler for quantitative radiotracer evaluation in bones and teeth, 458
TOTO, P. D., 1167 (Annot.)
Trace minerals, bivalent, effect on growth and glycolysis of oral acidogens, 1149
Tranquilizing drugs in oral surgery, 14 (Abs.)
Transplantation, homogenous, of hamster second molars from young to adult animals, 36 (Abs.)
Transplanted tooth germs, blood vessels and hormones in castrates bearing, 37 (Abs.)
TRAUTZ, O. R., 78 (Abs.)
TRIEGER, N., 21 (Abs.)
TUCKER, J. L., 87 (Abs.)
Tumors, carcinoma of tongue in mice, 950 (Annot.)
malignant lymphoma, 21 (Abs.)
TURESKY, S., 64 (Abs.), 119, 1115
TURNER, N. C., 788

ULCERS, oral, experimental production of, in rat, 38 (Abs.)
Ultrasonics, cleaning and sterilization of dental instruments, 43 (Abs.)
high speed rotary operative dental procedure, biology of. I. tooth volume and pulpal pathology, 67 (Abs.)
pulpal reactions, human, following 20,000 and 150,000 r.p.m. cavity preparations, 68 (Abs.)
Ultrastructural observations on oral mucosa, 18 (Abs.)
Urinary calculi formation, lack of effect of fluoride, 69 (Abs.)
fluorine levels, children and adults, 601

VAN HAERINGEN, W., 125
Vascularization of the condylar cartilage, 753 (Abs.)
VENKATESWARLU, P., 69 (2 Abs.)
Vertical dimension, a study, 44 (Abs.)
VISEK, W. J., 264
Vitamin A, effect of deficiency upon experimental carcinogenesis, 19 (Abs.)
effect of deficiency in periodontal structures of rat molar, 61 (Abs.)
hypervitaminosis, 8 (Abs.)
some effects on growth and development of jaws and teeth, 752 (Abs.)
Vitamin C, ascorbic acid, role in fluoride retention, 72 (Abs.)
effect of deficiency on condylar osteoclasts, 80 (Abs.)
localization in dental tissues in scorbutic and non-scorbutic guinea pigs, 6 (Abs.)
Vitamin E-depleted rats, curative effect of alpha-tocopherol and protein, 732

WACHTEL, L. W., 59 (Abs.)
WAGNER, M. J., 70 (Abs.), 72 (Abs.)
WAIT, D. M., 214
WALLIS, C. P., 748 (Abs.)
WALTER, E. L., 175
WALTHALL, C. D., 35 (Abs.)
WANNAMACHER, R., 292
WANTLAND, E. M., 12 (Abs.), 949 (Annot.)
WANTLAND, W. W., 12 (Abs.), 949 (Annot.)
WARKANY, J., 8 (Abs.)
WARNER, B. W., 35 (Abs.), 80 (Abs.)
WATERMAN, J. M., 47 (Abs.), 49 (Abs.)
WATSON, B. F., 53 (Abs.)
Wax patterns, dimensional change in, during setting of gypsum investments, 351
Waxes, indirect inlay pattern, evaluation of their manipulation, 95 (Abs.)
WEBER, T. B., 28 (Abs.), 902
WEBSTER, R. C., 16 (Abs.), 649
WEDDERBURN, D. L., 756 (Abs.)
WEINMANN, J. P., 54 (Abs.), 492
WEINSTEIN, I., 48 (Abs.)
WEISBERGER, D., 21 (Abs.)
WELLS, H., 55 (Abs.)
WENTZ, F. M., 46 (Abs.)
WESSINGER, G. D., 206
WESTERDON, E. M., 749 (Abs.)
WETHERELL, J. A., 754 (Abs.)
WHEATCROFT, M. G., 59 (Abs.), 96 (Abs.)
WHITE, E., 87 (Abs.), 89 (Abs.), 547
WHITE, W. L., 13 (Abs.)

Whitlockite and octocalcium phosphate, formation and stability, 78 (Abs.)
occurrence in dental calculus, 749 (Abs.)
WILKE, E., 53 (Abs.)
WILKINSON, E. G., 136
WILLETT, N. P., 930
WILLIAMS, G. C., 162
WILLIAMS, N. B., 51 (Abs.), 951
WINER, R. M., 76 (Abs.)
WINQUIST, D. L., 12 (Abs.), 949 (Annot.)
WISOTZKY, J., 27 (Abs.)
WOLLMAN, D. H., 805
WOODS, R. W., 865
WRIGHT, D. E., 756 (Abs.)
WYNN, W., 33 (2 Abs.), 66 (Abs.)

X-RAYS, *see* Radiation and radiography

YALE, S. H., 6 (Abs.)
YEN, P. K-J., 458
YOON, S. H., 25 (Abs.)
YUKTANANDANA, I., 46 (Abs.)

ZACH, L., 67 (Abs.)
ZACKIN, S. J., 38 (Abs.)
ZANDER, H., 44 (2 Abs.), 61 (Abs.), 1035
ZENEWITZ, J., 581, 590
ZITA, A. C., 76 (Abs.)

No pain—use of XYLOCAINE HCl assures effective anesthesia that carries patients safely beyond the pain threshold of even the most difficult dental procedures.

No strain—patients are more relaxed, less apprehensive, more responsive, and appreciative of your care and consideration.

ASTRA PHARMACEUTICAL PRODUCTS, INC., WORCESTER 6, MASSACHUSETTS, U. S. A.

XYLOCAINE® HCl INJECTABLE SOLUTION

(brand of lidocaine*)

for better doctor-patient relationship

*U. S. Patent No. 2,441,498

Designed for Every Dental Practitioner

THE PHYSIOLOGICAL FOUNDATION of DENTAL PRACTICE

by

L. L. LANGLEY, Department of Physiology, University of Alabama School of Dentistry, Medical College of Alabama, Birmingham, Ala.,

and

E. CHERASKIN, Department of Oral Medicine, University of Alabama School of Dentistry, Birmingham, Ala.

2nd Ed. 587 pages, 192 illustrations.

Price, **$9.75.**

Pointed toward the dental profession this book takes up those phases of physiology which are of prime importance to the practicing dentist. This eliminates the necessity of wading through cumbersome tomes on physiology to dig out what the practitioner actually needs.

The physiology of the nervous system is first considered and comprises almost a third of the entire work. This is justified on the contention that a dentist is concerned more intimately with the nervous system than with any of the other systems. On the other hand, the dental practitioner has only a cursory interest in the excretory mechanisms. For this reason, a consideration of this subject comprises but a relatively small section. The other subjects comprising the study of physiology have been likewise treated according to their relative importance to the dental practitioner.

Order today from

THE C. V. MOSBY CO.

3207 Washington Blvd.
St. Louis 3, Mo.

Date _____

THE C. V. MOSBY COMPANY
3207 Washington Blvd.
St. Louis 3, Mo.

Gentlemen: Send me Langley-Cheraskin "THE PHYSIOLOGICAL FOUNDATION OF DENTAL PRACTICE," priced at $9.75.

☐ Attached is my check. ☐ Charge my account.

Dr. _____

Street _____

City _____ Zone ____ State _____

DR-12-58

HELP US KEEP THE THINGS WORTH KEEPING

Each of us wants peace for his own precious reasons. But peace costs money. Money for strength to keep the peace. Money for science and education to make peace lasting. And money saved by individuals to keep our economy strong. Each Bond you buy helps provide this money—helps strengthen America's Peace Power. Are you buying *enough*?

HELP STRENGTHEN AMERICA'S PEACE POWER

BUY U. S. SAVINGS BONDS

The U. S. Government does not pay for this advertising. The Treasury Department thanks, for their patriotic donation, The Advertising Council and this magazine.

This New Guidebook Shows You "How," "When" and "Where" to Apply Techniques

Nagle-Sears
DENTAL PROSTHETICS
Complete Dentures

By RAYMOND J. NAGLE, D.M.D., Dean, New York University College of Dentistry; Professor, Denture Prosthesis, New York University College of Dentistry; Diplomate, American Board of Prosthodontics; and VICTOR H. SEARS, D.D.S., Formerly Professor, Prosthetic Dentistry, New York University College of Dentistry; Diplomate, American Board of Prosthodontics.

With the collaboration of SIDNEY I. SILVERMAN, D.D.S., Assistant Professor and Director of Graduate and Postgraduate Prosthodontics, New York University College of Dentistry; Diplomate, American Board of Prosthodontics.

Practical, easy to read and to abstract, DENTAL PROSTHETICS is a thorough, basic guidebook covering complete dental prosthetics—demonstrating "how," "when" and "where" to apply techniques. It relates the principles of clinical care to the basic sciences in a way that will not become out-dated when the next new impression material comes along.

In the first part of the book, the basic biological sciences are presented as applied sciences with emphasis placed upon the developmental point of view in terms of phylogeny, embryology and the growth and development of tissues. In the second part of the book, the successive phases of treatment for complete dentures are presented: diagnosis, impression procedures, occlusal records and esthetics. Abundantly illustrated with 360 photographs and drawings, DENTAL PROSTHETICS uses photographs and drawings in a very effective way to give a three dimensional quality to tissues as they move through space in relation to dentures. In comparison with other books, it places more stress on function, i.e., mastication, respiration, speech and deglutition. In addition, newer understanding of tissue behavior in relation to histology, physiology, pathology and bio-physics makes this a book well worthy of a place in your personal collection.

Just published.

532 pages, 6¾" x 9¾", 360 illustrations. Price, $11.50.

At Your Favorite Bookstore or Order on 10 Day Approval From

The C. V. MOSBY Company

3207 Washington Boulevard, St. Louis 3, Missouri

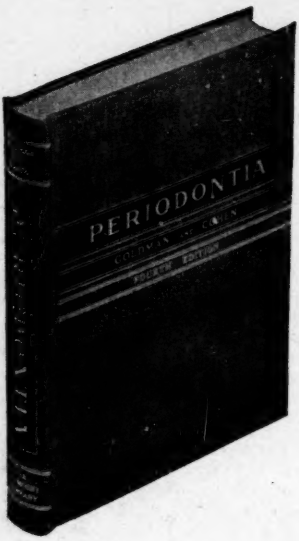
CHRISTMAS SEALS AT WORK!

A most important medical discovery during the past year was a blood test for the detection of tuberculosis.

Developed at Northwestern University under a research grant from the National Tuberculosis Association, it is now undergoi widespread trials. This was made possible by your purchase of Christmas Seals.

Since 1907, the work financed by Christmas Seals has helped cut the TB death rate 95%—but within the next 20 years almost 2,000,000 Americans will be afflicted with tuberculosis. So, send in your contribution today, please.

Buy and use Christmas Seals



You'll get that *"I Could Do That"* feeling from this study of

PERIODONTIA

The Art and Science of Examination and Diagnosis of Manifestations of Periodontal Diseases

Stimulating New Chapters in This Edition

- **The Role of Bacteria in Periodontal Disease**
- **Radiographic Interpretation**
- **Periodontal Disease in Children**
- **Gingival and Oral Manifestation of Infectious Dermatologic and Systemic Disease Origins.**

Just Published
4th ed. 535 pages,
6¾" x 9¾"
366 illustrations
Price, $12.50

By HENRY M. GOLDMAN, D. M.D., F.A.C.D., Director of the Riesman Dental Clinic, Beth Israel Hospital, Boston; Chairman of the Department and Professor of Periodontology, Graduate School of Medicine, University of Pennsylvania; and D. WALTER COHEN, D. D.S., Assistant Professor of Oral Medicine and Oral Histopathology, School of Dentistry, University of Pennsylvania.

Correlating etiology and diagnosis to the underlying tissue changes, the new 4th edition of PERIODONTIA presents the anatomy, histology, physiology and pathology of the periodontium in such a fashion that it is easy to understand the basic principles involved. Because the presentation is so vivid and clear, the reader is left with the "I could do that" feeling.

Unlike earlier editions of this book which included treatment, this revision presents just the principles and procedures of examination and diagnosis of periodontal manifestations and supports these procedures with information concerning the investing and supporting structures of the teeth. Because therapy is so thoroughly and adequately discussed in a separate volume, PERIODONTAL THERAPY by Goldman-Schluger and Fox, it is left out of this edition.

Emphasizing that each case is an entity in itself, the book shows that it is of vital importance to have a complete picture in order to institute proper treatment. The reader is led carefully from the anatomy, histology and physiology of the periodontium through the pathology to a consideration of the multiple etiologic factors.

Mark and Mail This Coupon Today!

Order today from

THE C. V. MOSBY CO.

3207 Washington Blvd.
St. Louis 3, Mo.

Date_____

The C. V. Mosby Company
3207 Washington Blvd., St. Louis 3, Missouri.

Gentlemen:

Please send to me on 10 day approval a copy of Goldman-Cohen PERIODONTIA priced at $12.50. I understand that if I am not completely satisfied, I can return the book within 10 days and my money will be completely refunded.

☐ Payment enclosed ☐ Charge my account

Doctor _____

Address _____

City _____ Zone _____ State _____

DR-12-58

CONTENTS FOR NOVEMBER—DECEMBER, 1958 (*Cont'd*)

Chemical and Physical Studies of Enamel From Human Teeth. III. Specific Gravity, Nitrogen Content, and Histologic Characteristics of Opaque White Enamel. B. R. Bhussry, Rochester, N. Y. ---------- 1054

The Measurement of the Abrasion of Human Teeth by Dentifrice Abrasives: A Test Utilizing Radioactive Teeth. R. J. Grabenstetter, R. W. Broge, F. L. Jackson, and A. W. Radike, Cincinnati, Ohio ---------- 1060

Alteration of Blood Flow to the Teeth. II. Its Effect on Dental Caries and Relation to Salivary Gland Structure and Function in the Rat. Harold E. Brewer and Joseph C. Muhler, Bloomington, Ind. ---------- 1069

Dental Caries in the Molar Teeth of Rats. I. Distribution of Lesions Induced by High-Carbohydrate Low-Fat Diets. Paul H. Keyes, Bethesda, Md. ---------- 1077

Dental Caries in the Molar Teeth of Rats. II. A Method for Diagnosing and Scoring Several Types of Lesions Simultaneously. Paul H. Keyes, Bethesda, Md. ---------- 1088

Normal Development of Tooth Buds Cultured In Vitro. William Lefkowitz and Patricia Swayne, Columbus, Ohio ---------- 1100

A Histochemical Study of the Attached Gingiva in Pregnancy. Samuel Turesky, Beverly Fisher, and Irving Glickman, Boston, Mass. ---------- 1115

Emergence of the Permanent Teeth in Pima Indian Children. Albert A. Dahlberg and Renée M. Menegaz-Bock, Chicago, Ill. ---------- 1123

Cobalt-Induced Polycythemia. I. Clinical and Hematologic Observations. Clark N. Eid and Robert J. Gorlin, Minneapolis, Minn. ---------- 1141

Effect of Deletion of Bivalent Trace Minerals on Growth and Glycolytic Activity of Oral Acidogenic Microorganisms. Samuel Dreizen, William Niedermeier, Jo G. Dreizen, and Tom D. Spies, Chicago, Ill. ---------- 1149

Osteogenic Potential of Calcium Hydroxide and Other Materials in Soft Tissue and Bone Wounds. David F. Mitchell and Govind B. Shankwalker, Indianapolis, Ind. 1157

Calcification and Esterase Localization in Developing Dentin. W. B. Savchuck and M. S. Burstone, Bethesda, Md. ---------- 1164

Research Annotation

Oral Lactobacillus Acidophilus Growth Inhibiting Activity of Human Globulin. Thomas L. Grisamore and Patrick D. Toto, Chicago, Ill. ---------- 1167

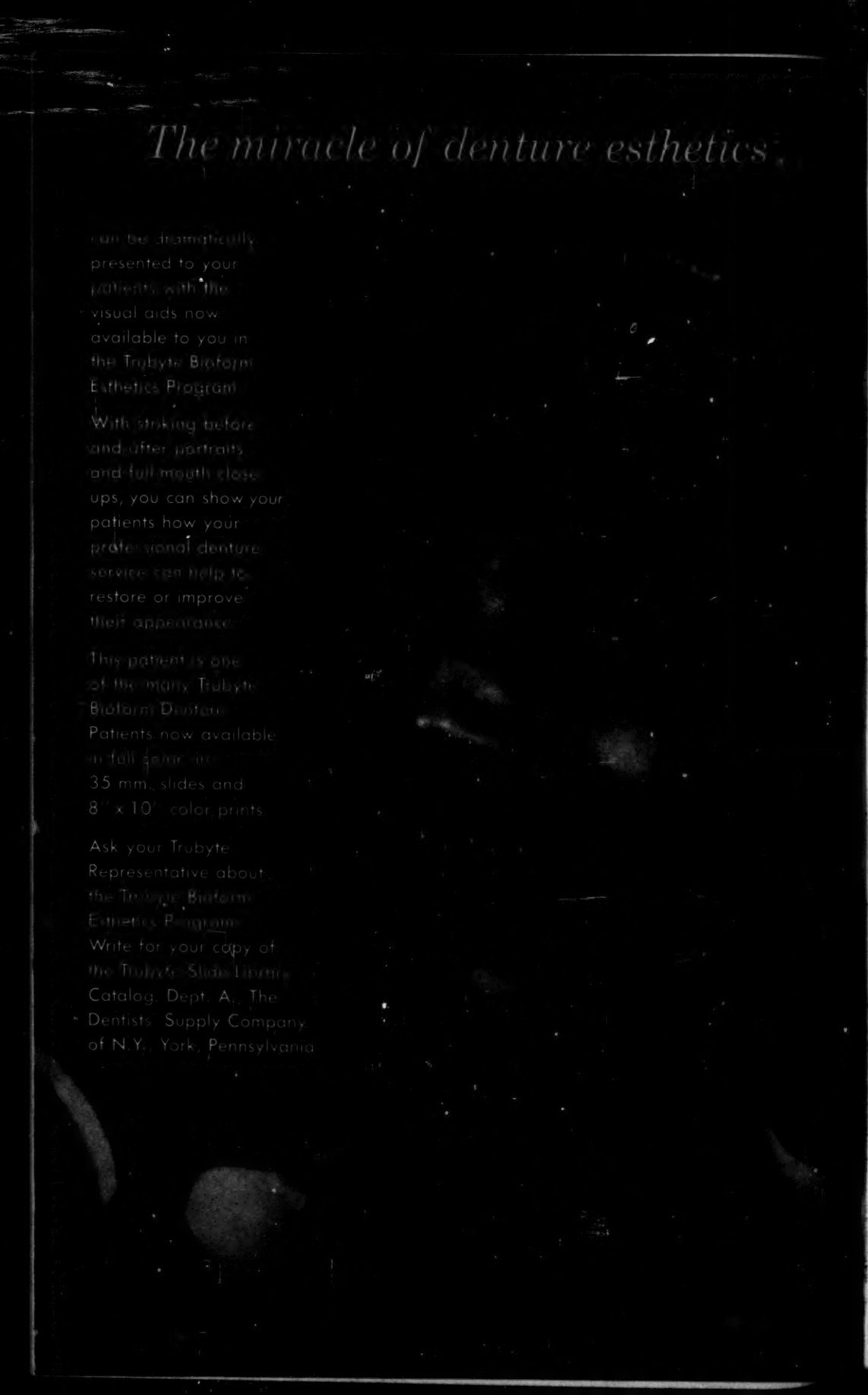
Index

Index ---------- 1169

The miracle of denture esthetics

can be dramatically presented to your patients with the visual aids now available to you in the Trubyte Biotform Esthetics Program.

With striking before and after portraits and full mouth close ups, you can show your patients how your professional denture service can help to restore or improve their appearance.

This patient is one of the many Trubyte Biotform Denture Patients now available in full color in 35 mm. slides and 8" x 10" color prints.

Ask your Trubyte Representative about the Trubyte Biotform Esthetics Program. Write for your copy of the Trubyte Slide Library Catalog. Dept. A, The Dentists' Supply Company of N.Y., York, Pennsylvania.